THE AMERICAN JOURNAL OF PHYSIOLOGY

EDITED FOR

THE AMERICAN PHYSIOLOGICAL SOCIETY

CONTENTS

PAGE

THYROID STUDIES. II. CHANGES IN THE THYROID GLAND PRODUCED BY FECAL EXTRACTS. *H. P. Rush and L. T. Jones* 1

MECHANISM OF PUPIL INEQUALITY FOLLOWING BILATERAL SECTION OF THE CERVICAL SYMPATHETIC. *Joseph Byrne and Carl P. Sherwin* 9

THE SENSITIVITY OF THE SMALL INTESTINE AT DIFFERENT LEVELS TO INTERNAL PRESSURE. *J. W. Crane and V. E. Henderson* 22

ON THE SPECIFIC DYNAMIC ACTION OF PROTEINS IN THIN AND FAT INDIVIDUALS (DOGS). *Rebekah Gibbons* 26

THE FUNCTION OF THE PERIPHERAL NEURONES IN THE CONDUCTION OF IMPULSES IN THE SYMPATHETIC NERVOUS SYSTEM. *Arie Querido* 29

EFFECT OF DOUBLE ADRENALECTOMY ON THE BLOOD COAGULATION TIME IN CATS. *O. W. Barlow and M. M. Ellis* 58

ON THE INFLUENCE OF TEMPERATURE ON THE TONUS WAVES OF THE TURTLE AURICLE. *Jayme R. Pereira* 68

DAY TO DAY VARIATIONS IN BASAL METABOLISM OF WOMEN. *Roberta Hafkesbring and Mary E. Collett* 73

LEAD STUDIES. IV. BLOOD CHANGES IN LEAD POISONING IN RABBITS, WITH ESPECIAL REFERENCE TO THE STIPPLED CELLS. *J. Albert Key* 86

A STUDY OF TESTICULAR VOLUME CHANGES. *C. E. King and R. M. Oslund* 100

VASECTOMY ON DOGS. *Robert M. Oslund* 111

ON THE RELATION OF LABYRINTHINE AND RETINAL EXCITATIONS IN THE RABBIT. *S. S. Maxwell and George F. Pilz* 118

DIURESIS IN THE SHEEP. CONCENTRATION OF URIC ACID AND UREA BY THE EXCRETORY MECHANISM OF SHEEP AND RABBIT COMPARED. *J. Lucien Morris, Vernon Jersey and Charles T. Way* 122

THE TOXICITY OF THE "ACETONE BODIES." I. ACETONE ADMINISTERED INTRAVENOUSLY. *Benj. H. Schlomovitz and E. G. Seybold* 130

ELECTRICAL STUDIES IN MAMMALIAN REFLEXES. IV. THE CROSSED EXTENSION REFLEX. *Alexander Forbes and McKeen Cattell* 140

STUDIES IN GASTRIC SECRETION. I. THE PSYCHIC SECRETION OF GASTRIC JUICE UNDER HYPNOSIS. *Arno B. Luckhardt and Robert L. Johnston* 174

STUDIES ON INTESTINAL INHIBITORY REFLEXES. *C. E. King* 183

SOME OBSERVATIONS ON THE EFFECT OF INCREASED AIR MOVEMENT AND WATER INTAKE ON THE DOG DURING AN EXPOSURE TO HIGH ENVIRONMENTAL TEMPERATURE. *Frederick B. Flinn* 194

THE EFFECT OF THYROXIN AND ITS ACETYL DERIVATIVE ON AMPHIBIANS AND MAMMALS. *W. W. Swingle, O. M. Helff and R. L. Zwemer* 208

VOL. LXX—NO. 1

Issued September 1, 1924

BALTIMORE, U. S. A.

1924

Entered as second-class matter, August 18, 1914, at the Post Office at Baltimore, Md., under the act of March 3, 1879. Acceptance for mailing at special rate of postage provided for in section 1103, Act of October 3, 1917. Authorized on July 5, 1918

THE AMERICAN
JOURNAL OF PHYSIOLOGY

VOL. 70 SEPTEMBER 1, 1924 No. 1

THYROID STUDIES

II. Changes in the Thyroid Gland Produced by Fecal Extracts

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Received for publication May 21, 1924

Remedi (1), using separately diphtheria and tetanus toxin injections directly into the thyroid gland, demonstrated changes in its structure. Farrant (2) injected various toxins intraperitoneally including both tetanus and diphtheria toxins. He found that after administering diphtheria toxin a hyperplasia always resulted. After further investigation (3), including the histological examination of 700 thyroid glands, part of them derived from humans and part from experimental animals, he has classified a great number of diseases as to the degree to which they affect the thyroid. As a result of experimental work he also concludes that it is the toxin from the microörganism and not the microörganisms themselves which produce this change. Rogers and Garnier (4) obtained changes in the thyroid following infections. McCarrison (5) reported his exhaustive work showing that the feeding of fecal extracts and residues to rats produced thyroid changes. He (6) further states that he found a strong tendency for the enlargement of the thyroid in animals which drank largely of water contaminated by feces. This work was carried out on rats, goats and humans by the administration of the sediment of water that contained excreta and waste material from goiter districts. These enlargements so produced disappeared if the water containing these sediments was boiled. Intestinal antiseptics also proved of value in treating goiter in these regions, according to this author. Later McCarrison (7) found that rheumatisn, rheumatic arthritis and malaria are repeatedly accompanied by thyroid changes. Burget (8) produced thyroid hyperplasia in rats kept under unhygienic conditions. Marine and Lenhart (9) found goiter in fish which were kept in contaminated water. The condition automatically disappeared after proper sanitary procedure. Bircher (10) fed feces with cooked rice to rats and obtained definite thyroid changes. Hart and Steenbock (11) fed normal sows a diet contain-

ing no roughage, thereby producing poorer elimination and increasing intestinal putrefaction. Congenital goiter appeared in all of the offspring.

On the other hand Basinger (12), repeating the work of Remedi, found no evidence from which to infer that diphtheria or tetanus toxins produced thyroid changes. Burget (8), using cats and giving the emulsion of feces from both goitrous and non-goitrous individuals by stomach tube, found no change in the thyroid. Whipple and Dragstedt (14), (15) both have shown that very little, if any, fecal toxin is absorbed from the normal digestive tract. Kalkus (16) showed that when no iodine deficiency was present, goiter never developed in his animals. Marine, Kimball, Lenhardt and Rogoff (17) have prevented the occurrence of goiter in school children by administering minimal amounts of iodine. Thus it is seen that the conflicting evidence so far reported leads to no definite conclusion.

In the light of such an existing controversy, it seemed to us that perhaps some additional information might be obtained by the further use of certain toxins.

In these experiments rabbits were used as the experimental animals. They were kept in clean cages. Both experimental and control animals were kept at the same room temperature since Mills has shown that extreme limits may affect the thyroid. The amount of light was also roughly controlled (13). All animals had practically the same diet and were governed according to ordinary laboratory methods. Lastly, we were very careful to keep all iodine from the room in which the animals were housed.

The toxin used was extracted from rabbit feces with physiological saline. This fecal extract was prepared fresh daily. Fresh feces were used in order to keep the concentration of the extract as constant as possible. A known amount was taken and ground in a small meat grinder. Four times this quantity of normal saline was added and the mixture allowed to macerate twenty-four hours. It was then run through gauze, filter paper and lastly the Berkefeld filter to insure a bacteria-free preparation. Absolute sterility was not maintained throughout as we found that by using fresh solutions and thoroughly washing the final container each day no infections resulted. An extract so prepared produced death in rabbits when 10 to 12 cc. per pound of body weight were injected intraperitoneally. The possibility that death might have been produced by shock was eliminated by a similar administration of the same quantity of normal saline in control rabbits.

Most animals used in series I and II were females of approximately the same weight. In the first and second series of animals a partial thyroidectomy (one lobe) was performed before any toxin was administered. After weighing, the tissue was fixed in Orth's fluid. Sufficient time was always

allowed for recovery of the animal before any experimental procedure was imposed. Each rabbit was weighed twice a week. The belly of each was shaved and injections made six days a week. The first injection was 10 cc. of toxin, this being increased by 10 cc. on each succeeding day until the dosage reached 40 cc., which dose was maintained. This amount was established as the approximate maximal sublethal dose, by previous experiments. An autopsy was performed on the animals after death. The remaining lobe of the thyroid was removed, weighed and placed in

TABLE 1

GROUP	RABBIT	SEX	DAYS	AMOUNT OF TOXIN	DOSES	AVERAGE DOSE	WEIGHT		WEIGHT THYROID		GENERAL APPEARANCE OF GLAND
							Lost	Gain	First lobe	Second lobe	
				cc.		cc.	lbs.	lbs.	mgm.	mgm.	
I	61	F	2	10	1	10	¼		95	100	Mild hyperplasia
	71	F	3	30	2	15	¾		145	170	Mild hyperplasia
	66	F	4	60	3	20	1		340	275	Mild hyperplasia
	69	F	30	Control rabbit			0	0	50	55	Normal
II	68	F	8	180	6	30	¾		175	270	Hyperplasia
	60	F	8	180	6	30	½		?	?	Hyperplasia
	65	F	16	500	14	35	1¼		105	105	Marked hyperplasia
	67	F	30	1020	26	39	2¼		150	140	Marked hyperplasia
	34	F	30	Control rabbit				½	60	75	Normal
III	1	F	77	308	46	6	¼				Mild hyperplasia
	3	F	75	333	46	7	¾				Mild hyperplasia
	4	M	72	420	46	9	0	0			Mild hyperplasia
	2	F	77	Control rabbit*			1¾				Mild hyperplasia
IV	44	F	57	842	35	24	1¾				Degeneration
	45	F	71	769	45	17		½			Marked hyperplasia
	46	F	71	593	39	15		½			Marked hyperplasia
	55	F	71	523	45	11		1½			Hyperplasia

* Control rabbit 2 became pregnant during the experiment.

the fixing fluid. The tissue was then embedded and sections, 5 microns in thickness, were cut and stained with hematoxylin and erythrosin.

In the third series, the preliminary partial thyroidectomy included but one-half of one lobe. An average dose of 7 cc. of toxin was given daily, with now and then periods of a few days of rest. At the end of the experiment the animals were killed and autopsied. The remaining thryoid tissue was prepared and sectioned.

In the fourth series unoperated animals were injected with moderate doses ranging from 11 to 24 cc. daily, a few rest days being given to insure

complete absorption. After six to eight weeks of experimentation a partial thyroidectomy was performed on each rabbit of this group. But little toxin was given after this operation and the animals were killed within four to six weeks. The thyroid tissues from operation and also from autopsy were prepared for histological examination. Table 1 gives a summary of the experimental conditions.

The animals showed characteristic symptoms following each injection. These were marked if the injections were large and mild or absent if the injections were small. Abdominal distress seemed present. Respirations invariably became greatly increased in rate for a time. Within fifteen to thirty minutes the animals would begin to show signs of depression, appearing to be in a state of shock. Muscular weakness developed, the rabbits being unable to sustain their weight on their legs. The respiratory rate slowed and became subnormal. The animals almost always defecated

TABLE 2

HOUR	TEMPERATURE	
	Sub lethal dose	Lethal dose
1	102.2	103.0
2	99.5	99.8
3	100.1	98.5
4	100.3	97.0
5	99.5	Died
6	97.5	
7	97.6	
8	97.9	
9	98.2	
10	99.0	
24	102.0 Recovery	

and urinated shortly after the injections. The temperature became subnormal following each injection and all animals showed an extremely low temperature before death if a lethal dose was given. (No. 65 had a terminal peritonitis and temperature of 105.4, which was caused by intestinal perforation from hypodermic puncture.) Table 2 shows a characteristic temperature reaction, the normal temperature of rabbits being approximately 102.5°. Three control rabbits (nos. 81, 83, 84), which were given the same amounts of normal saline intraperitoneally, showed none of the above symptoms. All animals except the controls lost weight.

The normal rabbit thyroid is quite uniformly constant in its histological appearance. The cells vary from a flat to a low cuboidal. The nuclei are slightly angular or round, very distinct, and stain moderately deep. The colloid content is moderate in amount and stains rather deeply, and is for the most part uniform. The follicles vary in size from small to

medium, the shape being round or ovoid and the edges are quite constantly regular. Projections into the follicles by epithelial cells are not observed. The intrafollicular cells are not of great quantity. Masses of solid epithelial cells are uncommon but not lacking. The stroma is scant and vacuolization of the colloid is sometimes observed, but not in any large degree.

The specimens of thyroid removed at autopsy from series I showed marked absorption of colloid. The remaining colloid stained poorly, was intensely vacuolated, and somewhat granular in appearance. The acini were unequal in size, some being completely empty. The epithelium was high cuboidal to columnar. The intervesicular tissue was not increased. The blood vessels showed dilatation. Two types of epithelial cells were seen; those with large, clear, granular nuclei and clear cytoplasm, and those with smaller, dark nuclei and granular cytoplasm, staining as normal cells. Processes from the cells lining each acinus seemed to extend into the colloid so that the cell boundaries were indistinguishable in places. A few acini showed definite desquamation of epithelium into the lumen.

The specimens taken from series II presented a similar but more advanced picture. There was a greater degree of intervesicular cell growth and formation of new acini. Epithelial projections into the larger alveoli were abundant. The epithelium was columnar with a more granular and reddish cytoplasm. Both types of nuclei were in abundance, many of the darker staining nuclei having the appearance of mitotic figures. An occasional typical mitotic figure was seen. Polychromatic granules were present in the cytoplasm of many of the lining cells of the acini at the poles of the cells nearest the lumen. Other signs of hyperplasia described in the thyroids of series I were present. An occasional alveolus was seen in which the colloid was completely absorbed, leaving only scattering fragments of cells. Cytolysis was apparent in some of the epithelial cells. Mononuclear phagocytes were seen in the intervesicular spaces.

In the final specimens of thyroid removed from series III, these animals being subjected to small doses over a long period of time, variations from the normal were also observed. The cells appeared more cuboidal, although some flat cells were seen. The colloid seemed to be in less amount and the follicles showed a slight irregularity. They varied in size from small to medium large and in some cases quite a large size was reached. Vacuolization of the colloid was not common. Hyperplasia of the parenchyma was moderate. The interfollicular cells were more prominent than in the normal and in places masses of epithelium could be seen. Slight hypertrophy of the cells was noted. The stroma appeared normal in amount. All changes were moderate in degree.

The first specimens of thyroid tissue examined from series IV were those removed during the height of toxin administration. These sections showed the colloid to be decreased in amount. Vacuolization was common

and in some sections quite pronounced. The follicles varied in size from small to large and were very irregular in appearance. Projections of cells were noted in the acini. The epithelium varied from high cuboidal to columnar showing area of some hyperplasia and in some cases hypertrophy. Areas of masses of cells were seen quite frequently. The interfollicular tissue appeared to be increased. The nuclei for the most part were of one type but in some sections the two types previously described were seen. Examination of the sections made from the tissue removed at autopsy showed a different picture. The colloid was more abundant in amount and vacuolization, while not absent, was decidedly less. The follicles were more regular in appearance and did not vary as much in size. There were but few projections noted in the acini. The cells were cuboidal, varying from high to low. Areas of masses of cells were seen but these were infrequent. Hyperplasia was present in some sections but not pronounced. The interfollicular tissue was increased above the normal amount but less so than in the preceding sections. The nuclei were all of one type, being clear and staining deeply.

The results of these experiments would indicate that thyroid tissue reacts to fecal extract injections. The first reaction seems to be an increased activity. This is manifest in the more acute condition, as is shown in series I and II, by a pronounced colloid absorption with a beginning hyperplasia and in some cases a mild hypertrophy. The process progresses if the intoxication continues or becomes more severe. This is evidenced by the small amount of colloid and the more pronounced hyperplasia shown in the rabbits whose injections covered a longer period of time. The greater the amount of fecal extract given, the greater were the changes observed in the thyroid, providing quantities closely approaching the lethal dose were not given. The results obtained from series IV are quite conclusive. In these animals the thyroid showed decidedly more change during the height of fecal extract injections than they did at the

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Fig. 1. I: Section removed from rabbit 45 during the period of injections. Note colloid absorption, moderate hypertrophy and hyperplasia.

II: Section removed from rabbit 45 about three weeks following section in I. Note decrease in colloid, marked hypertrophy of cells and variation of follicles.

III: Normal thyroid section from rabbit 71.

IV: Section from rabbit 71 after toxin injections. Duration, three days. Note vacuolization of colloid and two types of nuclei.

V: Normal thyroid section from rabbit 4.

VI: Section from rabbit 4 after toxin injections. This animal had small amounts over a long period of time. Note the increase in cells and the irregularity of follicles. Colloid absorption is noted in one location.

VII: Section from rabbit 67 which was taken following the administration of large doses of toxin over a period of one month. Note degenerative changes present.

Note: The magnification of I, II and VII is the same, of III and IV the same and of V and VI the same.

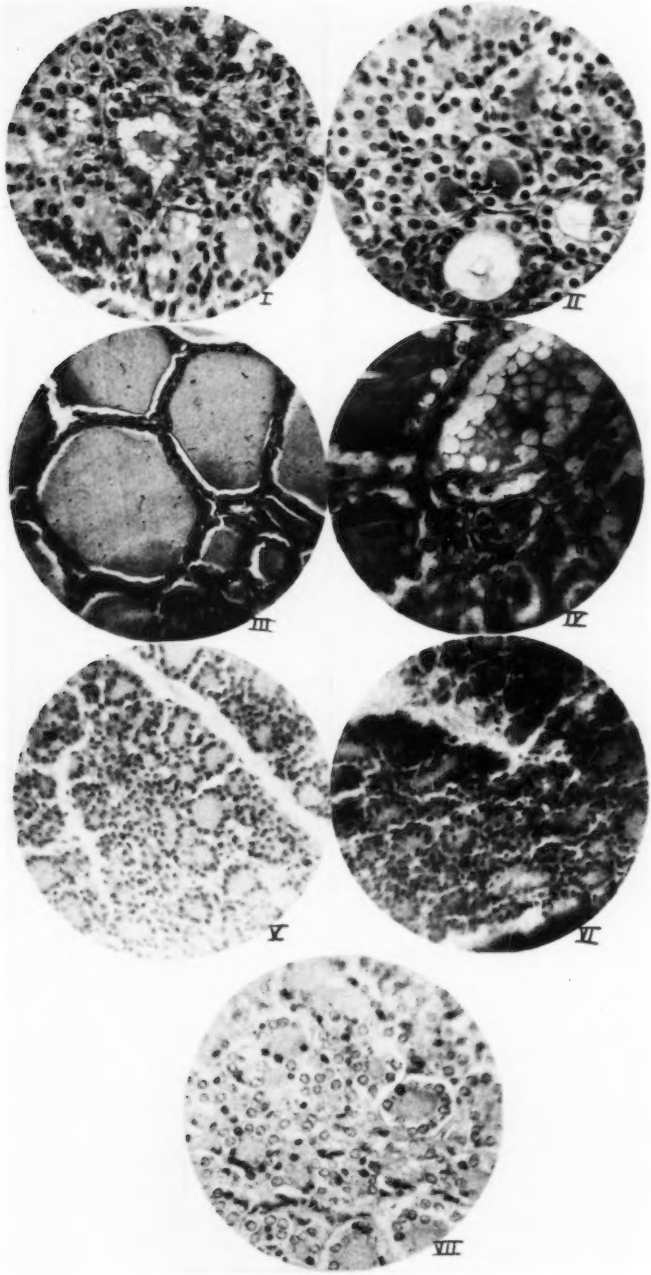

Fig. 1

end of the experiment when the amount of toxic material which they were receiving was much less. Therefore it would seem as though some intoxications, at least, call forth an increased thyroid activity. Whether this increased activity denotes an increased thyroid output or a detoxication function for the thyroid we are not prepared to say, but experiments now being conducted appear to favor the former idea.

All rabbits used in these experiments did not show the typical increased activity described but some (67–44) gave a picture of degeneration. While these two animals are not sufficient from which to draw conclusions, it would seem that a heavy dosage, one nearly approaching the lethal dose, could not be tolerated long by the rabbits. The thyroid activity was apparently unable to meet the demand and the thyroid succumbed to the intoxication which had been produced. Degenerative changes were seen in these glands which were characterized by the disappearance of colloid, cytolysis of the epithelial cells, parenchymatous degeneration, destruction of nuclei and evidences of inflammatory reaction.

CONCLUSIONS

1. Fecal extract when injected intraperitoneally into rabbits causes an increased activity of the thyroid gland as evidenced by colloid absorption, hyperplasia and hypertrophy.

2. The increase in activity of the gland seems to be roughly comparable to the amount of toxin given.

3. It would seem as though the thyroid breaks under the strain of large doses of toxin and undergoes degenerative changes as evidenced by cytolysis and varying degrees of inflammatory changes.

4. This response so far has shown itself to be merely a physiological increase in the gland.

BIBLIOGRAPHY

(1) Remedi: Sperimentale, 1902, lvi, 500 (quoted by Basinger).
(2) Farrant: Lancet, 1913, ii, 1820.
(3) Farrant: Brit. Med. Journ., 1914, v, 470.
(4) Rogers and Garnier: Compt. Rend. Soc. de Biol., 1898, l, 873.
(5) McCarrison: Ann. Trop. Med., 1911, v, 87; 1912, vi, 1.
(6) McCarrison: Indian Journ. Med. Res., 1914, ii, 183; 1914, ii, 214.
(7) McCarrison: The thyroid gland in health and disease, New York, 1917.
(8) Burget: This Journal, 1917, xliv, 492.
(9) Marine and Lenhart: Bull. Johns Hopkins Hosp., 1910, xxi, 95.
(10) Bircher: Deutsch. Zeitschr. f. Chir., 1911, cxii, 368.
(11) Hart and Steenbock: Journ. Biol. Chem., 1917, xxxiii, 313.
(12) Basinger: Journ. Infect. Dis., 1917, xx, 131.
(13) Aschoff: Endocrinol., 1922, vi, 919 (abst.).
(14) Whipple: Johns Hopkins contributions to medicine, 1921.
(15) Dragstedt: Journ. Exper. Med., 1917, xxv, 421.
(16) Kalkus: St. Coll. Washington, Agric. Exper. Sta. Bull. 156.
(17) Marine, Lenhart, Kimball and Rogoff: Western Reserve Pub.

MECHANISM OF PUPIL INEQUALITY FOLLOWING BILATERAL SECTION OF THE CERVICAL SYMPATHETIC

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Received for publication May 29, 1924

After section of one cervical sympathetic (cat) the homolateral is the smaller pupil but if the other cervical sympathetic be divided at any time during the few days following the first operation the pupil on the side first operated on is found to be larger than its fellow at all times except when the animal is absolutely quiet. Schafer (1) has recently called attention to this fact, mentioning the 5th day following the first operation as the time when the second operation best shows the phenomenon. It should be kept in mind that sympathetic section not only affects the pupil but also the nictitating membrane, the degree of protrusion of the eye ball (proptosis, etc.), and the caliber of the blood vessels of the interior of the eye (chorioid and iris) although in some animals, e.g., the rat and guinea pig, the proptosis seems to be more affected whilst in other animals e.g., the cat, the pupillary effects are more in evidence.

METHOD. The present studies are an attempt to elucidate the mechanisms of the Schafer and allied phenomena and as pseudo- and true paradoxical pupil phenomena play an important part in these mechanisms the reader is referred to papers by one of us (2), (3), (4) for the mechanisms and methods of study of these phenomena. In operations on the neck the skin incision was made in the median line, the rest of the operations following the usual route for exposure of the vago-sympathetic. As a preliminary to selection, the eyes of all animals were carefully studied and tested by intravenous injections of adrenalin. The measurements of the pupils, *p*, membranes, *m*, and palpebral fissures, *f*, were estimated and set down in millimeters the first of each set of figures representing the right pupil, etc., and the second the left pupil, etc.

EXPERIMENTAL FACTS: *Cat 1.* Cut right cervical sympathetic. On morning of 5th day, after adrenalin m ii, measurements were: *p* 7.0:7.5, *m* 0.5:4.5, (true paradoxical dilator effects absent in right pupil but present in right membrane). One hour after section of left sympathetic on 5th morning measurements were: *p* 6.0:4.0, *m* 3.0:4.0, *f* 6.0:5.0 and 4 hours later: *p* 6.0:4.5, *m* 3.5:4.5, *f* 6.0:5.0. These figures represented true as well as pseudo-paradoxical dilator phenomena in the right eye since the inequality of the pupils, etc., was greatly increased after adrenalin. On the

6th day measurements were about as on 5th and spinal cord transection between roots C i and ii diminished but did not abolish the inequality. Adrenalin continued to evoke true paradoxical phenomena in right eye.

Cat 2. Cut right sympathetic. On 3rd day adrenalin failed to elicit true paradoxical effects in right eye and 7 hours after left sympathetic section measurements were: *p* 2.5:2.0, *m* 4.0:4.0, *f* 6.0:6.5. On giving ether (early stage) measurements were: *p* 8.0:7.0, *m* 3.0:3.0, *f* 7.0:5.0 (pseudo-paradoxical phenomena in right pupil and fissure). Adrenalin failed to elicit true paradoxical phenomena in right pupil, membrane or fissure. Twenty minutes after removal of right superior cervical ganglion and ganglion nodosum and section of left vagus measurements were: *p* 9.0:8.5, *m* 3.0:4.0, *f* 9.0:7.5 (pseudo-paradoxical phenomena in right pupil, membrane and fissure). On 4th day 15 hours after removal of right superior cervical ganglion the measurements 1 minute after adrenalin were: *p* 8.0:4.0, *m* 2.5:4.0, *f* 9.0:5.0 (true paradoxical effects in right eye).

Cat 3. Procedures as in cat 2 for 1st and 3rd days. Paradoxical and pseudo-paradoxical phenomena found in right eye after section of left sympathetic. Ergotoxine in carefully graded doses abolished pseudo-paradoxical phenomena in right eye.

Cat 4. Procedures as in cat 2 for 1st and 3rd days. Ergotoxine failed to abolish true paradoxical hypersensitivity in right eye.

Cat 5. Right vago-sympathetic laid bare for one inch with as little manipulation of nerve as possible. On 3rd day measurements were: *p* 8.5:8.0, *m* 0.5:0.5, but on giving ether right soon became smaller than left pupil. Adrenalin failed to elicit true paradoxical phenomena in right eye. On this day, 9 hours after section of right and left sympathetic, measurements were: *p* 4.0:3.5, *m* 3.5:3.5 and adrenalin failed to elicit true paradoxical phenomena in right eye.

Cat 6. Stripped right vago-sympathetic as in cat 5. On 5th day measurements were: *p* 6.0:4.5, *m* 0.5:0.5, *f* 7.0:6.5 (pseudo-paradoxical phenomena in right pupil and fissure). On this day, after section of right and left sympathetic, measurements were: *p* 8.0:7.0, *m* 4.5:4.5, *f* 8.0:7.0. On 7th day right eye exhibited pseudo-paradoxical phenomena which were abolished on giving ether (late stage) when measurements stood: *p* 3.0:6.0, *m* 5.5:5.0, *f* 6.0:7.0. Adrenalin evoked marked true paradoxical phenomena in right eye.

Cat 7. Cut post ganglionic (superior) branches of right sympathetic. After section of left sympathetic, on 2nd day, measurements were: *p* 8.0:3.0, *m* 2.0:5.0 (true paradoxical phenomena in right eye).

Cat 8. Procedures as in cat 7 with same results.

Cat 9. Cut right post ganglionic fibers of sympathetic at 4:30 p.m. and at 9:35 a.m. on following day right and left sympathetics were cut; at 2:10 p.m. measurements were: *p* 2.5:2.0, *m* 5.0:4.5, *f* 8.0:7.5. At 2:20 p.m. adrenalin evoked true paradoxical phenomena in right eye measurements 1 minute after adrenalin being: *p* 6.0:4.5, *m* 1.5:3.0, *f* 8.0:6.0.

Cat 10. Cut right post ganglionic branches. On 3rd day 10 cc. of serum of second sample of blood taken from cat 12 were injected intravenously but failed to elicit true paradoxical phenomena in right (sensitized) eye.

Cat 11. Cut left sciatic. On 4th day right was larger pupil (pseudo-paradoxical dilatation). On 6th day, 8 hours after section of right and left sympathetic, right was larger pupil, e.g., *p* 4.0:2.0, *m* 3.5:3.5. Blood taken from animal at this time showed glucose 0.192 per cent. On 8th day measurements were: *p* 9.0:7.5, *m* 2.0:2.0, *f* 8.0:6.5.

Cat 12. Cut left sciatic. On 5th day measurements were: *p* 6.0:5.0, *m* 2.0:2.5, *f* 5.5:5.0 (pseudo-paradoxical phenomena in right eye). Under ether, wink

reflex absent, measurements were: *p* 9.0:9.5, *m* 1.0:2.0, *f* 7.0:6.0. One hour after taking 20 cc. of blood from left femoral vein, measurements were: *p* 6.0:4.0, *m* 2.0:3.0, *f* 8.0:7.0 and adrenalin evoked true paradoxical phenomena in right eye. Six and one-half hours after taking blood from left femoral vein measurements were: *p* 8.0:7.0, *m* 0.0:0.5, *f* 8.0:7.0. Blood (20 cc.) was again taken from left femoral vein. Seven minutes later measurements were: *p* 3.5:3.0, *m* 0.0:2.0, *f* 7.0:6.0 and now adrenalin caused well-marked true paradoxical phenomena in right eye. The first sample of blood contained 0.283 per cent of sugar measured as glucose and the second 0.178 per cent.

Cat 13. Cut left sciatic. On 5th day with animal quiet and untouched measurements were: *p* 9.0:10.0, *m* 0.5:0.5, *f* 8.0:8.0. On slightest excitement right became the larger pupil. Adrenalin failed to elicit true paradoxical phenomena in right eye. Five hours after cutting the right vago-sympathetic and the left sympathetic, measurements were: *p* 5.0:3.5, *m* 4.0:4.5, *f* 5.5:5.0 (pseudo-paradoxical effects in right eye). Under ether measurements were: *p* 7.0:8.0, *m* 4.0:4.5, *f* 5.0:5.0. Adrenalin in two trials failed to elicit true paradoxical phenomena in right eye but after intravenous administration of 25 cc. of 5 per cent glucose solution adrenalin evoked well-marked true paradoxical phenomena in right eye.

Cat 14. Through median skin incision dissection was made on right side of neck similar to that required for cutting vagus or sympathetic nerve but without injuring or manipulating the carotid sheath. Immediately after operation measurements were: *p* 8.0:7.0 (pseudo-paradoxical dilatation). On 2nd day measurements were: *p* 7.0:6.0, *m* 1.0:1.0, *f* 8.5:8.0 (pseudo-paradoxical phenomena in right eye). On 4th day when quiet right was smaller pupil but became the larger on approach of a dog, measurements being: *p* 9.0:7.5, *m* 0.0:0.5, *f* 9.0:7.5 (pseudo-paradoxical phenomena in right eye). At 9:10 a.m. (4th day) right and left cervical sympathetics were cut and 6½ hours later measurements were: *p* 4.0:3.5, *m* 4.0:4.5, *f* 5.5:5.0 (pseudo-paradoxical phenomena in right eye). Under ether right became smaller pupil and adrenalin evoked true paradoxical phenomena in right eye but $CaCl_2$ (20 cc. of $\frac{m}{8}$ solution) failed to evoke paradoxical constriction in right pupil. At 4:25 right was larger pupil but following spinal cord transection between roots C i and ii it became the smaller, measurements at 4:43 being: *p* 2.5:3.0, *m* 3.0:2.5, *f* 5.0:6.0 (pseudo-paradoxical phenomena in right eye abolished by cord transection). After the cord transection adrenalin evoked true paradoxical phenomena in right eye.

Cat 15. Opened carotid sheath and stripped vago-sympathetic with minimum of manipulation. On 2nd and 4th day pseudo-paradoxical phenomena in right eye, measurements on 2nd day being: *p* 7.0:6.0, *m* 0.0:0.5, *f* 8.0:7.5. On 4th day right and left cervical sympathetics were cut and 7½ hours later measurements were: *p* 6.0:4.5, *m* 2.5:3.5, *f* 5.0:4.0 (pseudo-paradoxical phenomena in right eye). Adrenalin ¾ of 1 drop of 1:1000 solution now failed to elicit true paradoxical phenomena in right eye but after intravenous injection of 20 cc. of $\frac{m}{8}$ solution of Ca Cl_2 the right eye exhibited paradoxical constriction phenomena the measurements, e.g., at 5:15 being: *p* 2.5:3.0, *m* 3.5:1.5, *f* 3.0:3.5. After spinal cord transection between roots C i and ii the right was generally the smaller pupil although at times it became the larger. Adrenalin m i of 1:1000 solution evoked true paradoxical phenomena in right eye though these were easily exhausted. In death right was the larger pupil.

Cat 16. Cut right sympathetic and in doing so slightly stretched the vagus. On 2nd day left sympathetic was cut and 8½ hours later measurements were: *p* 6.0:4:5, *m* 1.5:3.0, *f* 6.0:4.5 (pseudo- and true paradoxical phenomena in right eye). Ophthalmoscopic examination showed marked constriction of the blood vessels of the

right fundus oculi compared with those of the left. Ether and adrenalin evoked true paradoxical phenomena in right eye. After Ca Cl_2 the right pupil was not as large, compared with the left, as it had been and now the administration of ether evoked unmistakable paradoxical constriction phenomena in right eye the measurements, with the wink reflex present, being: *p* 1.5:2.5, *m* 2.0:1.5, *f* 5.0:6.0. As animal recovered from ether pseudo-paradoxical phenomena supervened, measurements being: *p* 7.0:6.0, *m* 0.0:1.0, *f* 9.0:8.0. Cord transection between roots C i and ii abolished the pseudo-paradoxical phenomena but asphyxiation and adrenalin still evoked marked true paradoxical dilatation phenomena in right eye the measurements, just after m ii of adrenalin, being: *p* 13.0:11.5, *m* 0.0:0.0, *f* 13.0:11.5.

Cat 17. The right superior cervical ganglion and ganglion nodosum were removed and the left cervical sympathetic divided. Measurements 12 hours later were: *p* 1.0:1.5, *m* 4.5:4.0, *f* 3.0:4.5. Right and left sciatic nerves were now crushed. Five minutes later measurements were: *p* 2.0:3.0, *m* 4.0:3.0, *f* 2.0:2.5; 10 minutes later adrenalin failed to elicit true paradoxical phenomena in right eye. With the animal quiet, 3½ hours after the 2nd operation, measurements were: *p* 0.5:1.0, *m* 4.0:3.5, *f* 4.0:4.5 but after lifting animal from cage measurements were: *p* 2.5:2.0, *m* 4.0:4.5, *f* 6.0:5.0 (pseudo-paradoxical phenomena in right eye). These measurements were reversed in the deep stages of ether but were accentuated in the early stages. At this time adrenalin failed to elicit true paradoxical dilatation in right pupil although right membrane and fissure gave an unequivocal paradoxical response the measurements, 30 seconds after adrenalin, being: *p* 3.0:6.0, *m* 0.0:3.0, *f* 8.0:6.0. It was not until 8 hours after the 2nd operation that adrenalin elicited frank paradoxical dilatation of right pupil when measurements, 30 seconds after adrenalin were: *p* 8.0:6.0, *m* 0.0:0.0, *f* 11.0:9.0. At this time 20 cc. of $\frac{m}{8}$ solution of Ca Cl_2 elicited a trace of paradoxical constriction in right eye.

Cat 18. Right and left cervical sympathetics were cut and right sciatic crushed. On morning of 5th day ophthalmoscopic examination showed constriction of fundus vessels of left eye; measurements were: *p* 4.0:5.0, *m* 4.5:5.0, *f* 5.0:6.5 (pseudo-paradoxical effects in left pupil, fissure and fundus vessels); adrenalin failed to elicit true paradoxical phenomena in either eye. Left sciatic was now crushed and 1 hour later, in the constriction period after adrenalin, measurements were: *p* 4.0:7.0, *m* 0.5:0.5, *f* 10.0:9.0 (accentuated pseudo-paradoxical effects confined to left pupil). On 7th day measurements were: *p* 3.0:4.0, *m* 4.5:5.0, *f* 5.0:6.0 (pseudo-paradoxical effects in left pupil and fissure). After adrenalin the paradoxical dilatation effects were greater in the left pupil, membrane, fissure, and fundus vessels, the measurements in the constriction stage being: *p* 6.0:7.5, *m* 3.5:3.0, *f* 5.5:7.0 (accentuated pseudo-paradoxical effects in left eye).

Cat 19. Crushed right sciatic. On 7th day, with the animal quiet, measurements were: *p* 7.0:6.0, *m* 1.5:2.0, *f* 10.0:10.5. When animal was disturbed measurements were: *p* 6.0:7.5, *m* 1.5:1.0, *f* 10.0:10.0 left fundus vessels smaller than right (pseudo-paradoxical effects in left pupil, membrane, and fundus vessels). After adrenalin measurements stood: *p* 14.0:14.5, *m* 0.0:0.0, *f* 13.5:13.0, right and left fundus vessels about equal in caliber (true paradoxical effects absent in left eye). Left sciatic was now crushed and 9 hours later, just after adrenalin, measurements were: *p* 13.0:14.5, *m* 0.0:0.0, *f* 13.0:14.5 (true paradoxical effects in left eye). After cord transection between roots thoracic xiii and li the pseudo-paradoxical effects in left eye were abolished but adrenalin continued to elicit true paradoxical dilatation phenomena in left pupil, membrane and fissure; and $CaCl_2$ elicited in the left eye paradoxical constriction phenomena which were most marked as the animal died and after death. In one other animal similar results were obtained.

DISCUSSION AND SUMMARY. The earlier physiologists in studying the cervical sympathetic were occasionally embarrassed by effects observed in the contralateral eye. Thus Dogiel's observation that stimulation of the cervical sympathetic tended to cause contralateral pupillary constriction was confirmed by Schenk and Fuss (11) who showed, however, that the contralateral constriction was due to the consensual light reflex. Many of the earlier physiologists regarded constriction of the blood vessels of the iris as the chief mechanistic factor in pupil dilatation consequent on cervical sympathetic stimulation until Budge and Waller (12) showed that dilatation can occur without variation in the blood supply of the iris. Salkowski (5), however, showed that actual constriction of the intraocular blood vessels may accompany pupillary dilatation, and Langley and Anderson (13) gave it as their opinion that although arterial constriction may be a possible factor in pupil dilatation vascular changes have little influence on pupillary movements. It seems also that some of the earlier physiologists were familiar with a phenomenon of near kin to the Schafer phenomenon discussed in this paper. Compare Budge (8) who found that superior cervical ganglionectomy was followed by greater paralytic phenomena (pupillary constriction, etc.) than simultaneous section of the contralateral cervical sympathetic although under certain circumstances after the lapse of 24 hours the pupil on the ganglionectomized side became larger than its fellow. These findings of Budge were fully corroborated by those in cat 17. The ophthalmoscopic findings in cats 16, 18 and 19 point to constriction of the blood vessels of the iris as a possible adjuvant of the hyperfunctioning pupil dilator effectors in both the pseudo- and true paradoxical sympathetic phenomena. In addition to the pseudo- and true paradoxical effects, conditioned fundamentally by the first sympathetic section, other factors must be considered as possibly playing a mechanistic rôle in the Schafer and related phenomena. One such factor is activation or inhibition of the endocrinous glands causing alterations in the blood relating to sugar content, hydrogen ion concentration, adrenalin and kindred substances. Alterations of any one of these constituents is capable of influencing the appearance of both pseudo- and true paradoxical phenomena. Compare the effects of direct injury of the thyroid gland, or of interference with its blood or nerve supply as an incident of the operations for section of the sympathetic nerves. Direct gross injury of the thyroid and of its vessels and nerves may be safely excluded in our experiments as the possibility of such injury was foreseen and carefully guarded against. The influence of the primary and secondary operations upon sugar content, etc., of the blood was tentatively studied in cats 11 and 12 but the conclusions reached have no definite significance. In cat 13 the influence of glucose in facilitating the elicitation by adrenalin of true paradoxical phenomena was strikingly

shown. Compare Loewy (6) and Loewy and Rosenberg (7) who found that pancreatectomy facilitated the appearance of paradoxical dilatation in a sensitized pupil. Byrne (unpublished experiments) repeated Loewy's experiments and found that the hyperglycemia incidental to the pancreatectomy was one of the main factors in facilitating the elicitation of the paradoxical phenomena. The decentralized neurones of the superior cervical ganglion are another factor to be reckoned with in the mechanism of the Schafer and allied phenomena since the usual incubation period of 6 or 7 days for true paradoxical phenomena after cervical sympathetic section becomes reduced to 23 or 24 hours after superior ganglionectomy. Still another factor must be considered. Byrne (9) has shown that after injury of one vagus in the neck paradoxical pupil constriction may be elicited in the homolateral eye. This points to the vagus as containing afferent proprioceptive paths which impinge upon the related efferent paths presumably at the constrictor center in the oculomotor nucleus. Injury of the vagus incidental to the operation for sympathetic section may, therefore, be a possible factor in causing pupil dilatation by reducing the afferent flow of vagal constrictor impulses. In cats 15, 17 and 19 the demonstration of paradoxical constriction phenomena points to such injury of the vagus as a possible contributing factor in the mechanism of the Schafer phenomenon. After removal of one superior cervical ganglion, as after section or injury of its post ganglionic fibers, Byrne (unpublished experiments) found that the incubation period during which adrenalin fails to elicit true paradoxical phenomena averages under ordinary conditions between 23 and 24 hours. After section of one cervical sympathetic the incubation period under ordinary conditions was found to average about 6 or 7 days although under certain conditions, e.g., great excitement, fear, etc. it was found that the incubation period might be reduced to 3 or even 2 days. After section of one sciatic nerve the incubation period for true paradoxical phenomena was found to average from 10 to 12 days whereas, after section of the cervical or cranial sensory nerves the incubation period averaged from 6 to 8 days (2), (3). It should be borne in mind that unilateral somatic lesions below the level of the umbilicus are followed by true and pseudo-paradoxical phenomena in the contralateral eye whereas unilateral lesions above that level are followed by true and pseudo-paradoxical phenomena in the homolateral eye (2), (3), (4). Pseudo-paradoxical phenomena seem to have no definite incubation period and may be seen almost at any time after lesion of the afferent nociceptive paths (4). They may be present in an eye in which adrenalin may evoke true paradoxical phenomena (9) and they tend to disappear when the animal is perfectly quiet but reappear on the slightest disturbance. Our experiments fully corroborate Schafer's observation. The 5th day after section of one sympathetic falls very near the end

of the incubation period for true paradoxical phenomena. In fact in cat 1 the administration of ether, as well as adrenalin, elicited true paradoxical effects in the membrane though not in the pupil (potential paradoxical pupil dilatation) and 7 hours after the 2nd operation both pseudo- and true paradoxical phenomena were exhibited by the right eye. In this animal as spinal cord transection between roots C i and ii reduced the pupil inequality, etc., and as adrenalin continued to elicit true paradoxical effects, it was concluded that the reduction was mainly the result of abolition of the pseudo-paradoxical component. Compare Byrne (4). In the Schafer phenomenon, therefore, as encountered about the 5th day, both true and pseudo-paradoxical phenomena seem to be the main mechanistic factors. Cat 2 shows that the Schafer phenomenon may be observed on the 3rd day when it seemed to be preponderately, if not wholly, due to the supervention of pseudo-paradoxical phenomena since adrenalin failed to elicit true paradoxical phenomena 7½ hours after the 2nd operation. That true paradoxical phenomena were a possible latent factor in cat 2 seems to be supported by cat 16 in which the Schafer phenomenon was present on the 2nd day and adrenalin elicited true paradoxical phenomena on that day 8½ hours after the second operation. Indeed it may be concluded that the Schafer and allied phenomena may be observed at almost any time after the first operation and that in the early periods after the first operation the supervention of pseudo-paradoxical phenomena is the main factor whereas, after the lapse of one or two days, true paradoxical phenomena add their quota which may altogether over-shadow the pseudo-paradoxical effects. In cats 3 and 4 ergotoxine abolished the pseudo-paradoxical factor presumably by paralyzing the sympathetic myoneural junctions. In cat 4 ergotoxine failed to abolish the true paradoxical factor.

The shortening of the period of incubation for true paradoxical phenomena found in cats 1, 2, 4 and 16 was undoubtedly the result of the 2nd operation although the rôle of the cutting of the 2nd sympathetic did not impress us as of any mechanistic significance other than by equalizing the efferent flow of sympathetic impulses on both sides of the neck and thereby permitting the true and pseudo-paradoxical phenomena to become perceptible in the eye on the side of the 1st operation. In cats 2, 9 and 17 it was found that the 2nd operation shortened considerably the incubation period for true paradoxical phenomena after removal of the superior cervical ganglion or section of its post ganglionic fibers. In cats 5 and 6 in which at the first operation the vago-sympathetic trunk had been merely exposed the Schafer phenomenon was readily demonstrated after the second operation, performed on the 3rd and 5th day respectively, in which the right and left sympathetic were simultaneously divided. In cat 5 the phenomenon was present on the 3rd day and

seemed to be wholly due to the supervention of pseudo-paradoxical effects since adrenalin failed to elicit true paradoxical phenomena. In cat 6 in which the phenomenon was present on the 5th day true paradoxical phenomena may have been a factor as adrenalin evoked well-marked true paradoxical phenomena on the 7th day.

The findings in cats 5 and 6 in which, at the first operation, neither sympathetic nerve was injured and in which at the 2nd operation both cervical sympathetics were divided, raised the question as to whether the Schafer phenomenon might not be primarily the result, in part at least, of the incidental operative lesions rather than of the sympathetic section as such. In cats 11 and 13 the phenomenon was demonstrated on the 6th and 5th days respectively after section of one sciatic, the second operation consisting of section of both cervical sympathetics (cat 11) and of the left vago-sympathetic and the right sympathetic (cat 13). In cat 14 after making, through a median skin incision, an extensive dissection wound on the right side of the neck without interfering with the carotid sheath or its contents, the Schafer phenomenon was in evidence before and after section of both cervical sympathetics on the 4th day when both pseudo- and true paradoxical phenomena were present, the pseudo-paradoxical phenomena disappearing upon spinal cord transection between roots Ci and ii. As in this animal after the 2nd operation $Ca\ Cl_2$ failed to evoke paradoxical constriction it may be concluded that injury of the vagus afferents did not play a part in the mechanism. In cat 15, on the other hand, in which the primary operation consisted of destroying the carotid sheath, $Ca\ Cl_2$ evoked paradoxical constriction in the right eye. In this animal, therefore, it may be accepted that injury of the vagal afferents contributed its quota to the pupil dilatation, etc., observed before and after the second operation the main factors, however, being pseudo- and true paradoxical (dilator) effects resulting from lesions of the nociceptive paths at the 1st operation. In this animal (cat 15) cord transection between roots Ci and ii reduced the pupil inequality by abolishing the pseudo-paradoxical (dilator) component (4). In cat 16 in which the right cervical sympathetic was divided at the 1st operation and on the 2nd day, $8\frac{1}{2}$ hours after the second operation, in which the left sympathetic had been divided, the typical Schafer phenomenon presented itself. At this time ophthalmoscopic examination showed marked constriction of the fundus vessels of the right eye and, as $Ca\ Cl_2$ evoked paradoxical constriction in the right eye, and cord transection between roots C i and ii reduced the inequality of the pupils, whilst adrenalin continued to evoke true paradoxical phenomena, it was concluded that in this animal both pseudo- and true paradoxical effects (including constriction of the blood vessels of the iris) were present in the right eye and were supplemented by the dilator effects incidental to injury of the right vagus at the 1st

operation. The results in cats 11, 13, 14 and 15 pointed strongly to the operative lesions incidental to the 1st sympathetic section as playing at least a contributory rôle in the Schafer phenomenon, that rôle consisting of the induction, in the early days after the 1st operation, of pseudo-paradoxical phenomena and in the later days of true paradoxical phenomena both sets of phenomena in so far as they arose from the incidental operative lesions being the result of lesions of the cervical noci-ceptive nerves. The results in cat 12 in which on the 5th day after left sciatic section the Schafer phenomenon was elicited by the mere withdrawal of blood from the left hind leg (hemorrhagic shock) seemed to indicate that in the second operation, as in the first, other factors than the mere sympathetic section play an important contributory, if not indeed, the fundamental rôle. Thus in cat 17 in which after right superior cervical ganglionectomy and simultaneous section of the left cervical sympathetic, the Schafer phenomenon became manifest in the right eye $3\frac{1}{2}$ hours after crushing both sciatic nerves, i.e., $15\frac{1}{2}$ hours after the first operation (ganglionectomy, etc). The mechanistic rôle of the second operation in this animal seemed to consist mainly of traumatic shock. Compare the observation of Byrne and Einthoven (10) that during the acapneic shock incidental to pulmonic over-aeration no action currents could be detected in the cervical sympathetic during stimulation of the sciatic nerve. This seems to indicate that in conditions of shock the flow of efferent impulses over the cervical sympathetic tends to become checked and there is abundant evidence (Byrne, unpublished experiments) to show that such checking of the flow of efferent impulses is the fundamental proximate factor in the mechanism of true paradoxical ocular phenomena. In cat 17, as in cat 1, the incubation period for paradoxical effects in the membrane (withdrawal) and in Müller's muscle (proptosis) was shorter than for pupil dilatation proper. Compare cat 18 in which the pseudo-paradoxical effects resulting from crushing the right sciatic nerve were more marked in the pupil than in the membrane or fissure of the left eye. In this animal the pseudo-paradoxical effects in the left pupil were wholly the result of inhibition exerted at the constrictor center in the oculomotor nucleus by the hyperfunctioning primary affective neurones of the injured right sciatic. Compare Byrne (4). One contributing factor, therefore, in the Schafer phenomenon, viz., pseudo-paradoxical dilator effects, can be duplicated without injury of the cervical sympathetic at either operation. The findings in cat 19, in which both pseudo- and true paradoxical effects, the latter with remarkably shortened incubation period (7 days as against the average of 10 or 12 days), were obtained by 1st crushing one sciatic followed 7 days later by crushing of the other sciatic, seem to show conclusively that the full equivalent of the Schafer phenomenon may be obtained after primary and secondary lesions far removed from the

cervical sympathetic nerves. The effects of cord transection between roots thoracic xiii and li and of $CaCl_2$ injections seem to show conclusively that pseudo- and true paradoxical phenomena are the main factors in the mechanism with paralytic (dilator) effects from injury of the proprioceptive (constrictor) paths playing a subordinate rôle. In cat 17 the incubation period was shortened over 3 hours for true paradoxical dilatation by the second operation. In this animal the difference in the incubation period for paradoxical phenomena in the membrane and in Müller's muscle ($15\frac{1}{2}$ hours) as against that for pupil dilatation proper (20 hours) is to be accounted for presumably on the same grounds as the difference in the incubation period found after superior ganglionectomy (23 to 24 hours) and after section of the cervical sympathetic (6 or 7 days), viz., by the influence of the decentralized neurones of the superior cervical ganglion which, after section of the cervical sympathetic, continue to exert inhibitory influence on the dilator effector mechanisms in the iris for some days after the operation. It is probable that the sympathetic neurones found by many anatomists scattered along the course of the post-ganglionic (superior) fibers and in the ciliary body and iris also continue to exert some inhibitory influence on the effectors of the pupil, membrane and Müller's muscle. Such an assumption would account for the 24 hour incubation period after superior ganglionectomy and the further assumption that such post ganglionic neurones are functionally related for the most part to the dilator effectors and to a less extent to the retractor membranae and Müller's muscle affords a possible basis of mechanistic explanation for the difference in the incubation time (in cat 17) of the paradoxical pupil dilatation as against that for the membrane withdrawal and the proptosis. Explanation along similar lines would seem to hold also for the longer incubation period (10 to 15 days) which Byrne (unpublished experiments) found for paradoxical dilatation in the homolateral eye after hemitransection of the cervical spinal cord. In cat 17 the dilatation which appeared in the right pupil $15\frac{1}{2}$ hours after the ganglionectomy was in part due to the supervention of pseudo-paradoxical dilatation (including constriction of the blood vessels of the iris) and in part, perhaps, to paradoxical constriction of the blood vessels of the iris and possibly in part to dilatation consequent on injury of the right vagus. Compare cats 18 and 19. The attempt made by us to ascertain the changes, if any, in the blood incidental to the second operation, which might influence the appearance of paradoxical phenomena, true and pseudo-, is merely suggestive. The percentages of glucose found in the blood of cat 12 do not indicate that increased sugar content of the blood incidental to the 2nd operation is a factor. Care must be taken, however, in essaying interpretation on such a meager experimental basis. This aspect of the problem calls for experimental investigation on its own

account. The effort made to test the rôle of the blood, as affected by the second operation, by injecting the serum of the blood taken from cat 12 6½ hours after the 1st bleeding on the 5th day, into cat 10 in which the sympathetic effectors of the right eye had been sensitized must likewise be taken as merely suggestive. Though the effects on the sensitized eye were apparently nil it is realized that this aspect of the problem also calls for further investigation on its own account. It seems, therefore, that factors incidental to the first operation, other than the mere section of the cervical sympathetic, play important mechanistic rôles in the Schafer phenomenon even at as late a period as the 5th or 6th day. It seems also that the effects of the 2nd operation are incidental to traumatic shock rather than to the mere sympathetic section. The suspension of neural function which, as our experiments indicate, attends surgical shock brings to view an aspect of this condition which closely links it mechanistically with spinal shock so familiar to physiologists. Of particular interest to clinicians in this connection is the paradoxical hyperfunctioning of effector mechanisms as the result of suspension of neural functioning consequent on injury or inflammation of structures situated in remote parts. Compare, e.g., some of the blood vascular phenomena of surgical shock.

CONCLUSIONS

1. The Schafer phenomenon or its equivalent may be demonstrated as early as the 1st or 2nd day after the 1st sympathetic section.

2. It can be produced by lesions of the noci-ceptive paths in the neck incidental to the trauma of the first operation apart from the sympathetic section.

3. The effects of the first sympathetic section can also be duplicated by lesions of the noci-ceptive paths in regions far removed from the cervical sympathetic, e.g., by section or injury of one sciatic nerve.

4. The effects of the 2nd cervical sympathetic section, apart from the equalizing effect caused by abolition of the efferent sympathetic flow, may be duplicated by hemorrhage and by lesions in other parts of the body, e.g., sciatic section or injury.

5. The mechanism of the Schafer phenomenon consists mainly of the supervention of pseudo- and true paradoxical phenomena in the eye on the side of the first sympathetic section, the pseudo-paradoxical phenomena preponderating in the first day or so after the first operation and the true paradoxical phenomena a few days after the first operation. Besides these two main factors injury of the vagal afferents and pseudo- and true paradoxical constriction of the blood vessels of the iris possibly also play contributory mechanistic rôles.

6. The true paradoxical phenomena are the result in part of the first sympathetic section and in part of lesions of the cervical noci-ceptive paths incidental to the first operation as a whole.

7. The pseudo-paradoxical phenomena are the result exclusively of lesions of the noci-ceptive paths in the neck incidental to the first operation.

8. The sympathetic section at the second operation merely permits the pseudo- and true paradoxical phenomena induced by the first sympathetic section to become perceptible.

9. The longer incubation period for true paradoxical phenomena after section of the cervical sympathetic, as against superior ganglionectomy, seems to be due mainly to the temporary continuance of functional activity of the decentralized neurones whose bodies are located in the superior cervical ganglion.

10. After superior cervical ganglionectomy the incubation period for true paradoxical effects in the membrane (withdrawal) and in Müller's muscle (proptosis) is shorter than for pupil dilatation due probably to the relative preponderance in the post ganglionic paths of sympathetic neurones in functional relations with the *dilatator pupillae* as against neurones in functional relations with the retractor membranae and Müller's muscle.

11. The trauma of the second operation as a whole, including the effects of the anesthetic, and not the sympathetic section as such is the chief factor in shortening the incubation period for the true paradoxical phenomena the rôle of such trauma consisting of the induction of a condition akin to, if not identical with, spinal shock in which certain neural functions are in abeyance.

12. The equivalent of the Schafer phenomenon may be induced by first injuring one sciatic nerve followed about 7 days later by injury of the other sciatic.

13. The paradoxical hyperfunctioning of effector mechanisms as the result of suspension of the neural functioning incidental to injury or inflammation in parts remotely situated points to a reactionary phase of disease and injury worthy of the attention of clinicians. This is especially true of some of the phenomena observed in surgical shock.

14. Superficial examination failed to reveal in the blood after the second operation the presence in excess of substances (sugar, adrenalin-like substances, etc.) which might be contributory factors in shortening the incubation period for true paradoxical phenomena.

BIBLIOGRAPHY

(1) SCHAFER: Quart. Journ. Exper. Physiol., 1919, xii, 16.
(2) BYRNE: This Journal, 1921, lvi, 113.

(3) Byrne: This Journal, 1922, lix, 369.
(4) Byrne: This Journal, 1922, lxi, 93.
(5) Salkowski: Zeitschr. f. rat. Med., 1867, xxix, 167.
(6) Loewy: Arch. f. exper. Path. u. Pharm., 1908, lix, 83.
(7) Loewy and Rosenberg: Biochem. Zeitschr., 1914, lxvii, 323.
(8) Budge: Ueber die Bewegung der Iris, Braunschweig, 1855, 125.
(9) Byrne: This Journal, 1924, lxviii, 42.
(10) Byrne and Einthoven: This Journal, 1923, lxv, 350.
(11) Schenk and Fuss: Arch. f. Psych. u. Nervenkrank., 1899, lxxv, 110.
(12) Budge and Waller: Compt. rendus de l'Acad. d. Sci., 1851, xxxiii, 370.
(13) Langley and Anderson: Journ. Physiol., 1892, xiii, 554.

THE SENSITIVITY OF THE SMALL INTESTINE AT DIFFERENT LEVELS TO INTERNAL PRESSURE

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Received for publication May 31, 1924

The Trendelenburg (1) method of studying peristaltic movements has opened the way to a study of the sensitivity of the gut at various levels to the effects of pressure.

The technique employed was essentially that of Trendelenburg save in the following particulars: each piece of gut about 8 cm. long was tied over a separate cannula and immersed in the same bath of oxygenated saline solution, of pH of 7.5 prepared according to the method of Fleish (2). The three cannulae were connected to the one pressure bottle through a glass tube forking into three. A lateral offset near the pressure bottle was connected to a water manometer on which the pressure produced by raising the bottle could be read. A glass stopcock of large bore was inserted in each fork so that the pressure in any segment could be cut off independantly of the others. The pressure bottle was raised mechanically by clock work as described by one of us (3) elsewhere, so that the rate of pressure increase was constant. The movement of the pressure bottle was slow, approximately 30 mm. per minute. Each segment was attached by a thread to a light writing level so that longitudinal movements alone were recorded. Experience with the Trendelenburg method in which both circular and longitudinal movements are recorded, together with the use of guinea-pig gut which shows little or no rhythmic movements, enabled the observer under these conditions to be sure that the movements were peristaltic in nature.

Guinea pigs of about 400 to 500 gram weight were used. They were anesthetized with urethane. The greatest care was taken in removing the strips from the intestine. The mesentery was cut away with sharp scissors, each strip as removed was washed through with saline to clear out its contents, tied at the upper end and put into an oxygenated bath at 37°C. When the three strips were removed each was tied on to its cannula. The whole operation only required some four or five minutes. The experiment was usually not begun for a quarter of an hour. The strips were taken from lower duodenum, mid-jejunum and lower ileum, well clear of the colon. Great care had to be taken that the movement

of all levers was perfectly free from friction. Comparisons must be made only from fresh strips.

The observation made by Henderson (3) that the rate of movements differed according to the internal pressure was frequently confirmed. A certain pressure gives movements of maximal frequency while a slightly (2 to 3 mm.) lower pressure is effective in producing movements but more slowly and the movements are less frequent and fail sooner.

Two criteria of sensitivity may be employed, first, the critical pressure at which movements are produced in the different segments, and secondly, the time taken between the commencement of the rise in pressure and the manifestation of the first active shortening of the longitudinal muscle. Both these criteria were applied.

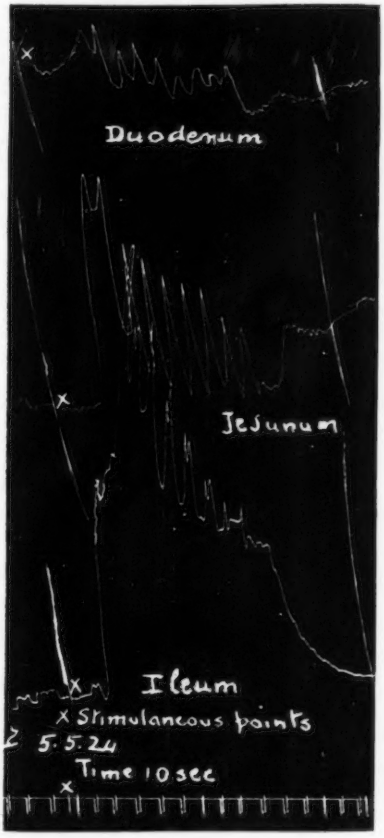

Fig. 1

Critical pressure. In some cases all three strips responded at the same pressure as nearly as could be estimated. Active strips often respond to a pressure of 8 mm. of water and it was impossible with the apparatus we were using to cut off the pressure in any strip accurately to the millimeter and consequently slight differences would be missed. In other cases where the pressure required was higher this could be done more accurately. Table 1 illustrates the type of result.

As may be seen from the above typical results selected from a great many, there is little difference in sensitivity between ileum and jejunum but that frequently the duodenum is less sensitive. This seems particularly to occur if the strips are fatigued.

Time relations. The time elapsing between setting the pressure bottle in motion and the first sharp upstroke of the recording lever was measured in 16 experiments in which clear-cut movements were obtained in all

three strips. As the pressure at which the pressure bottle stood when set in motion varied greatly and in consequence the length of time till the critical pressure was reached and the sharp upstroke was noted the figures do not denote any given period of latency and are merely comparative one with another. The average is duodenum 58.5, jejunum 56.1, ileum 54.9. On an average of these experiments the ileum shortened 1.2 seconds before the jejunum and 3.6 seconds before the duodenum. Cases occurred where there was no measurable difference, but as a rule there was a definite difference between ileum and duodenum. More frequently ileum and jejunum contracted at the same moment.

TABLE 1

		PRESSURE IN			REMARKS
		Duodenum	Jejunum	Ileum	
1.2.24	1	9	8	8	Duodenum only two waves at 9 mm., none at 8
	2	20	16	15	At 20 all strips good movements
	3	(8+)	8	8	None in duodenum
5.2.24	1	13	10	8	
	2	15	12	12	Movements in ileum more sustained
17.1.24	1	16	13	13	

In the experiment 5.5.24, figure 1, the times on the tracing shown are 19.5 seconds, 21.8 and 26 seconds. The average for a series of nine readings was 19.6, 21.7, 23.2 seconds.

The number of movements per minute made by the different strips varied greatly. For instance per minute

EXPERIMENT	PRESSURE	DUODENUM	JEJUNUM	ILEUM
	mm.			
No. 2. 12	18	15.4	8.5	9.7
13	13	8.5	8.5	8.5
2. (14)	8	4.2	2.8	
	16	10.7	9.5	6.5

On the whole, movements in the ileum appeared to be less frequent than in the other segments especially duodenum but were usually of much greater extent and often more resistant to fatigue.

Discussion. Alvarez (4) found in a study of the rhythmic movements of the gut that the movements were of a greater frequency in the upper portion than in the lower. He inferred that there was an axial gradient

in the musculature such that that of the upper portion of the intestine was more sensitive than in the lower and that the upper portion would respond more delicately to the peristaltic stimulus. This does not appear to be the case. Cannon and Murphy (5) showed that gentle manipulation of the gut for one-half hour in air inhibited the peristalsis but not the rhythmic movements. Whether the rhythmic movements are purely muscular is perhaps not decided though it is striking that the Magnus plexus free strip only showed rhythmic movements under abnormal conditions such as the presence of physostigmine while under more normal conditions it is quiescent. This, however, does not seem to be true of the strips presumably plexus free prepared by Gunn and Underhill (6). But the tissue must have been far from normal and even skeletal muscle under abnormal conditions shows movements. Such evidence as that submitted by Bayliss and Starling (7), Cannon and Murphy (5), and Magnus (8), Henderson (3) and others indicated that another factor than muscle alone is involved in peristalsis.

It is interesting also to note that the duodenum has larger quantities of fluid to handle than the ileum and that its villi are more active (9), and it would not appear surprising if it were less easily stimulated by internal pressure.

CONCLUSIONS

1. The stretching stimulus awakes a shortening and peristaltic response of the ileum at a lower pressure and more promptly than in higher segments.

2. There is less difference between ileum and jejunum than between ileum and duodenum.

3. The duodenum appears to tire more readily under its optimal stimulus than the other segments.

4. The peristaltic movement is repeated at shorter intervals than in the higher segments but is not so great in extent.

BIBLIOGRAPHY

(1) Trendelenburg: Arch. f. exper. Path. u. Pharm., 1917, lxxxi, 55.
(2) Fleisch: Arch. f. exper. Path. u. Pharm., 1922, xciv, 22.
(3) Henderson: This Journal, 1923, lxvi, 380.
(4) Alvarez: This Journal, 1914, xxxv, 177; 1915, xxxvii, 267; 1918, xlvi, 186.
(5) Cannon and Murphy: Ann. Surg., 1906, xliii, 512.
(6) Gunn and Underhill: Quart. Journ. Physiol., 1914, viii, 275.
(7) Bayliss and Starling: Journ. Physiol., 1914, viii, 275.
(8) Magnus: Pflüger's Arch., 1904, cii, 123, 349; 1904, ciii, 515; 1905, cviii, 1.
(9) King and Arnold: This Journal, 1922, lix, 96.

ON THE SPECIFIC DYNAMIC ACTION OF PROTEINS IN THIN AND FAT INDIVIDUALS (DOGS)

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Received for publication June 4, 1924

The following experiments were undertaken with the view of determining whether a difference in the degree of specific dynamic action of food might be a factor in the well-known phenomenon that of two animals of the same age and sex, on the same diet, and as nearly as possible the same amount of exercise, the animal with an hereditary tendency to leanness will remain thin, while the animal with an hereditary disposition to adiposity will put on fat. Analogous conditions (family traits toward adiposity or leanness) are also well known in man; and it is difficult to alter these predispositions to adiposity or leanness by diet, except when carried to extremes.

The specific dynamic action of 200 grams of ground calf heart from which all visible fat had been removed was tested on two male dogs of approximately the same age and weight. A thoroughbred greyhound was chosen as having an heredity tending to leanness, and a stocky mongrel (chiefly St. Bernard or mastiff) as an animal tending toward adiposity by racial inheritance (fig. 1). The weight of both dogs was the same, or between 29 and 30 kgm., and seldom differed more than 500 grams. The fat dog was consistently lighter. Both animals showed the usual autumn gain in weight, which was a little over 1 kgm. in each animal. The greyhound was 71 cm. tall at the shoulders and the fat dog 59. The average basal metabolic rate per 24 hours and per kilogram was 1242.2 and 41.8 calories for the thin, and 1035.5 and 34.9 calories for the fat dog. The basal metabolic rate of the thin dog was 19.9 per cent per 24 hours or 19.7 per cent per kgm. higher than that of the fat dog.

The basal metabolic rates of the animals were determined using a slight modification of Doctor Kunde's method. The animals were fed and tests taken in approximately 2, 3, 4, 6, 8, etc., hours after feeding. The height of the rise at exact hour intervals was estimated by plotting the rise per ten-minute period on graph paper using time and calories as coördinates. The total calorie increase was calculated from the area of the graph above the basal.

There are so many variables in tests of this type that only approximate results were expected. However, all tests show a greater specific dynamic

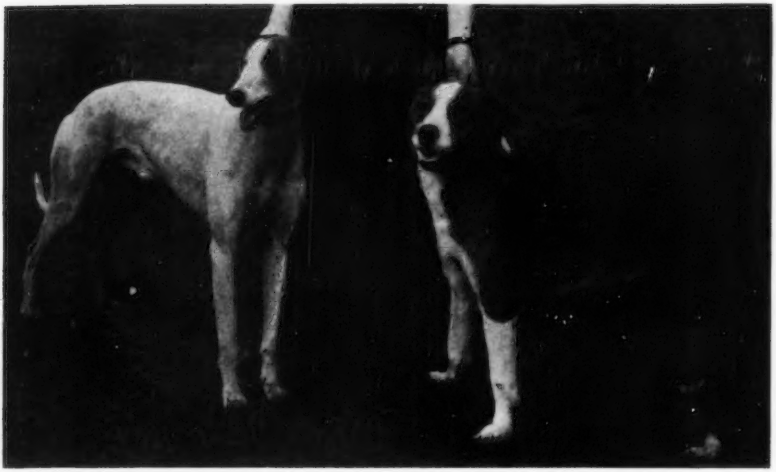

Fig. 1. Photo of lean and fat dog, showing conditions of the two animals that prevailed during the experiment.

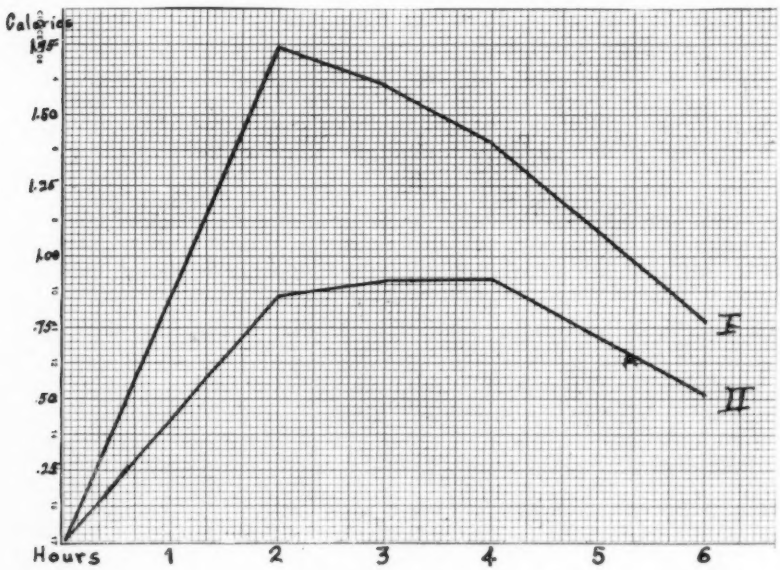

Fig. 2. Graphs of specific dynamic action of 200 grams of lean meat (heart muscle) in the lean dog (average of 27 experiments) and in the fat dog (average of 25 experiments).

action from meat in the case of the thin dog. The averages for 27 and 25 tests respectively on each dog are given in tables 1 and 2. While no final conclusions may be drawn from a single series of tests made on only one pair of animals, these tests show clearly a higher specific dynamic action of protein in the lean dog.

TABLE 1

Mean averages of specific dynamic action of 200 grams of heart muscle in thin and fat dogs

TIME AFTER FEEDING	HEIGHT OF RISE		TOTAL CALORIE INCREASE		PER CENT OVER BASAL	
	Thin dog	Fat dog	Thin dog	Fat dog	Thin dog	Fat dog
hours						
2	1.74	0.86	11.46	5.10	11.38	6.49
3	1.61	0.92	21.65	11.86	14.19	8.55
4	1.40	0.92	31.08	16.02	15.10	9.59
6	0.77	0.51	44.10	22.41	14.32	8.87

TABLE 2

Per cent by which figures for thin dog (table 1) exceed those for fat dog

TIME AFTER FEEDING	HEIGHT OF RISE	TOTAL CALORIE INCREASE	PER CENT OVER BASAL
hours	*per cent*	*per cent*	*per cent*
2	102.5	124.6	75.5
3	75.8	82.5	66.1
4	50.9	94.5	57.5
6	51.3	96.8	61.4

THE FUNCTION OF THE PERIPHERAL NEURONES IN THE CONDUCTION OF IMPULSES IN THE SYMPATHETIC NERVOUS SYSTEM

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Received for publication June 6, 1924

When studying the function of a nervous structure by recording its action on an end-organ, it is important that the exact conditions at this end-organ should be known so far as possible and that they should be uniform and reliable. For example, when we study the properties of a peripheral motor nerve by recording the response of a skeletal muscle, the number of structures involved in the experiment is known as well as the fact that every contraction in the muscle corresponds to an impulse or series of impulses that has travelled down the nerve trunk. It is easy to obtain graphic records of such muscles without much interference with circulation or exposure to air, but attempts to apply the same method to the autonomic nervous system meet with serious difficulties. The smooth muscle which constitutes the end-organ of the autonomic system is in almost every case so placed in the body that to connect it to a recording apparatus necessitates disturbance of natural conditions. How changes in the different structures of the preparation may influence the response obtained, how much danger there may be of simultaneous stimulation of the fibers belonging to the opposite type of innervation or to the antagonistic muscle-layer which is nearly always closely connected with that studied, what may be the influence of any ganglion-cells between the muscle-fibers, and how much the final response may be caused by the stimulation and how much by spontaneous activity, is not known. All these partially solved questions probably explain why, in the case of the autonomic system, the end-organ, i.e., smooth muscle, has attracted much more interest than have the innervating structures—except for their anatomical relations.

Any smooth-muscle system which presents relatively simple conditions is therefore of considerable value for the study of the autonomic system. The *membrana nictitans*, the third lid of the mammalian eye, probably contains the most simple system of this type in the mammalian body and offers the most advantageous conditions for recording

movements. Since there is no uniformity in statements concerning its ability to move actively, this point was investigated first.

THE END-ORGAN. *a. Histology.* A microscopic section through the nictitating membrane of the cat shows that this membrane may be regarded as a fold of the conjunctiva, enclosing a thin sheet of cartilage. This is the part visible in the intact animal. On the inner surface, towards the sclera, there is a small gland—the gland of Harder—and still further backward, at the root of the organ, is a muscle, 1.5 to 3 mm. long, which spreads like a fan through the membrane and is inserted in the outer layer of the sclera; this is apparently composed of smooth-muscle fibers between which non-medullated nerve fibers can be seen ending freely.

b. Motility. The membrane is capable of two movements: it can contract towards the median corner of the eye, or can come forward so that it more or less covers the eyeball. Some investigators, especially the zoölogists (1), (2), consider that these movements are purely passive, i.e., that contraction is caused by protrusion of the eyeball and consequent pushing away of the nictitating membrane; and that retraction of the eye, by the *retractor bulbae* muscle, permits the membrane to slide over the surface of the cornea.

Stimulation of the cervical sympathetic results not only in retraction of the membrane towards the median corner of the eye, but also in protrusion of the eyeball, as Krauss (3) has graphically demonstrated. If the eyeball is pierced, however, and its contents removed, so that it collapses and can no longer push away the membrane, retraction still takes place. In this case movement must certainly be active.

On stimulation of the III and VI cranial nerves inside the skull, the membrane spreads over the eye; simultaneously there is contraction of the external striated muscles of the eye.

Froëhlich and Loewi (4) consider that this movement of the membrane is active, not passive, because after application of curare, to paralyze the striated muscles of the eyeball, it still persists, and after the use of nitrites, which are supposed to paralyze the inhibitory sympathetic fibers, stimulation of the III and VI nerves causes movements of the eye without movement of the nictitating membrane. Neither of these experiments is conclusive; with regard to the first experiment, curare does not affect the layer of smooth muscle back of the eyeball, which might relax on stimulation of the abducent nerve and allow the eyeball to sink deeper into the orbit. And in the description of the second experiment there is no statement whether the cervical sympathetic was intact or not; if it is not severed, but is in control of the membrane, the eye simply moves without movement of the membrane, as it would in the intact animal. In his research on adrenalin Elliott (5) also seems to assume that

extension of the membrane over the eye is active, but he gives no evidence; Langley and Anderson (6) incline to the same view because they observed that stimulation of the oculomotor nerve can produce movements of the nictitating membrane without movement of the eyeball. The crucial experiment, however, shows that after removal of the contents of the eye, stimulation of the III and VI nerves has no effect on the membrane; thus, unless the orbit is filled with a mass, the membrane is unable to execute its extension. Extension therefore is passive.

Spontaneous movements of the membrane have not been observed. When nicotine is applied to the superior cervical ganglion retraction sometimes follows, but this cannot be called spontaneous. During stimulation the line of the graphic record has appeared several times rather irregular and undulating, with sudden changes of level instead of the usual smooth plateau, but activity without any stimulation has not been observed in any case.

c. Innervation. Langley (7), (8) found that the nerve-supply for the nictitating membrane originates at the level of the first and second thoracic segments. He also noted that, after application of nicotine to the superior cervical ganglion, stimulation central to the ganglion is without result, while stimulation peripheral to it is still effective. He therefore concludes that the termination of the pre-ganglionic fibers must be there. When nicotine is injected into the circulation, stimulation central to the ganglion is likewise ineffective, and peripheral stimulation is followed by full retraction. This is evidence that the only neurone junction in the nerve supply of the nictitating membrane is in the superior cervical ganglion.

In short, the nictitating membrane may be regarded as an organ containing smooth muscle without an antagonistic system, and supplied with nerves from only one part of the autonomic system; it contains no ganglion cells and shows no spontaneous activity. Its responses can be recorded without any interference with its blood supply or its natural state, and, as will be shown directly, its post- and pre-ganglionic nerve supply may be stimulated readily under known conditions.

That the nictitating membrane has not been more frequently used for the study of the properties of smooth muscle and of the autonomic nervous system is astonishing. So far as I know, Lewandowsky (9) who, in 1899, carried out a few experiments on the shape of the contraction curve and the latent period of the membrane, is the only investigator of this organ. Yet it offers opportunities for study of an autonomic end-organ under conditions more similar to those under which analogous studies have been made on striated muscle and its nerve supply than does any other autonomic end-organ in the body.

METHODS. All of the experiments reported were performed on cats; as an anesthetic urethane (25 per cent), chloralose or amytal was used. An incision was made in the median line from the upper edge of the sternum to the hyoid bone. The large anastomosis between the external jugular veins was ligated and severed. A tracheal cannula was inserted. The trunk of the vagus and sympathetic nerves was cut at the caudal end of the wound and freed for about an inch from the enveloping sheath of connective tissue. Silver electrodes (10 mm. apart and shielded according to Sherrington's method) were applied with the anode of the break towards the cut end of the nerve, for stimulating the pre-ganglionic fibers. The superior cervical ganglion was exposed, with care to avoid vascular injury; the post-ganglionic trunk was freed from connective tissue; and the vagus was severed close above the ganglion nodosum.

Before the main trunk of the sympathetic nerve enters the skull, there is a stretch about 6 mm. long in the middle of which fibers pass over towards the internal carotid artery, which here runs lateral to the nerves. Some of the fibers on the internal carotid supply the nictitating membrane. Pointed, hook-shaped silver electrodes 4 mm. apart were passed around the post-ganglionic trunk, one electrode on each side of the fibers running to the artery, and the anode caudad (fig. 1). A thin sheet of celluloid was then placed around the electrodes to insulate them from the surrounding tissues. After the electrodes were applied properly they were held in place by ligatures and the wound was closed. To permit reaching the ganglion without disturbing the electrodes a glass tube was sewed in the skin above the ganglion and closed with a piece of cotton.

The cat's eyelids were held apart by ligatures. The head was turned sideways in order to bring the movement of the nictitating membrane in the plane of the lever, and was securely fixed in a holder. Then a fine ligature was passed through the cartilage of the membrane and connected with a light lever, which magnified the movements six fold and provided a load of 5 grams. A glass writing-point attached to the lever by means of a small piece of celluloid, recorded the movements of the membrane on a smoked drum.

For stimulation, induction shocks were used from a small inductorium, manufactured by the Harvard Apparatus Company and calibrated for break-shocks by Martin's (10) method. The primary current was interrupted by a rotary interrupter similar to that described by Forbes (11); but instead of 6 segments—every other one insulated—2, 8 or 32 could be used. The brushes were so placed as to give 50 per cent closure. The number of revolutions was counted as Forbes described, by a magneto coupled on the same shaft. It is possible, with this instrument, to vary the frequency of interruptions from $1\frac{1}{4}$ per second to approximately 1000 per second, by changing the resistance in the armature of the motor-circuit.

Rates above 300 per second were not used, because above this frequency the shocks may be considerably weakened by "overlapping." Interrupting the primary circuit with lower frequencies yielded regular shocks of good size, as galvanometric control showed. The same switch that closes the stimulating circuit also closes a signal circuit by which the beginning and the end of stimulation are indicated. Changes in the stimulation were indicated by the same signal (middle line in records) by pressing a second key by hand. A double-throw double-pole switch in the secondary circuit allowed change without delay from one pair of electrodes to the other. Figure 2 shows schematically the principles of the set-up.

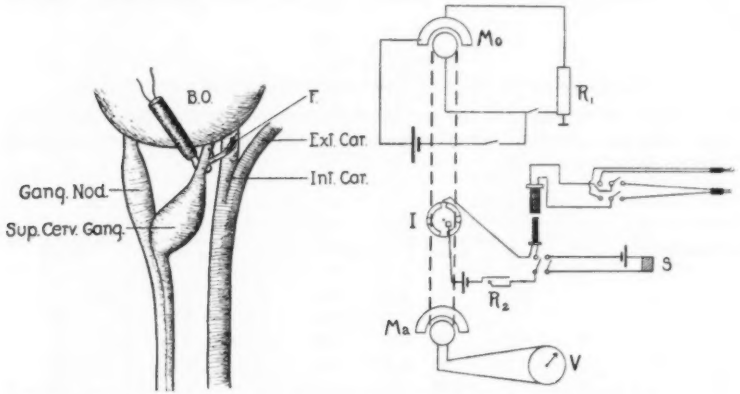

Fig. 1. Diagram of anatomical conditions. *B.O.* = bulla ossea. *F* = fibers, among which are those supplying the nictitating membrane (*nervus caroticus internus*)

Fig. 2. Wiring diagram. *Mo* = motor; *I* = interrupter; *Ma* = magneto; R_1 = resistance in motor-circuit; R_2 = resistance in stimulating circuit; S = signal; *V* = voltmeter.

OBSERVATIONS. *a. Influence of varying frequencies of post- and preganglionic stimulation.* In dealing with smooth muscle it is impossible to judge by the size of the response whether or not a stimulus is maximal, since nearly all strengths of stimulation, if long enough applied, will produce the same height of contraction. For the sake of brevity we will call a response as high as one that can be obtained with any stronger stimulus, a maximal response, according to the terminology used for the striated muscle. The more important point in these experiments was, however, to ascertain that the make-shocks would be too weak to be effective. Break-shocks were therefore used which had a strength, as the measurements of the action-currents in the pre-ganglionic fibers showed, just great enough to cause maximal responses. Since, under our conditions, according to Martin (12), make-shocks are one-eighth

as efficient, physiologically, as are break-shocks, their action under the conditions of these experiments can be neglected (cf. Forbes (13)). In some experiments, after stimulating for 1 second a pause of 20 seconds

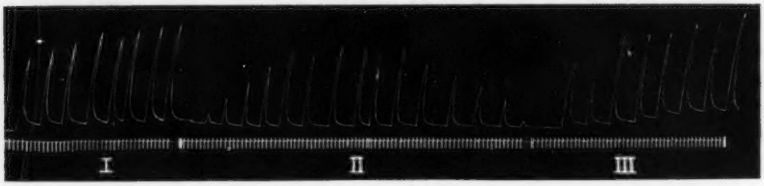

Fig. 3. Difference in the effects of pre- and post-ganglionic stimulation. Data in table 1.

TABLE 1 (cf. fig. 3)

Difference in the effect on pre- and post-ganglionic stimulation

Cat 2.6 kgm., 20 cc. urethane (25 per cent) by stomach tube, 1 hour later 2 cc. urethane intra-peritoneally. Usual procedure on left side. Strength of stimulus, 18 Z-units. February 1, 1924.

PRE-GANGLIONIC STIMULATION			POST-GANGLIONIC STIMULATION		
Series number	Number of observation	Frequency per second	Series number	Number of observation	Frequency per second
I	1	10	II	1	5
	2	25		2	10
	3	40		3	15
	4	80		4	20
	5	120		5	25
	6	160		6	40
	7	180		7	50
	8	200		8	60
				9	80
				10	100
				11	120
III	1	10		12	140
	2	25		13	160
	3	40		14	170
	4	80		15	180
	5	120			
	6	160			
	7	180			
	8	200			

was followed by stimulation for the same length of time but with a different frequency. With frequencies ranging from 5 to 260 per second, comparison was made of the heights of the contractions as obtained with both pre- and post-ganglionic stimulation. The strength of stimulus was kept constant during each series. The general aspect of a series of

records, obtained by stimulating both pre- and post-ganglionic fibers with different frequencies is shown in figure 3. The data of this experiment are given in table 1.

TABLE 2 (cf. fig. 4)

Difference in effect on stimulating the pre- and post-ganglionic fibers

Cat 2.0 kgm., 16 cc. urethane by stomach tube. Usual procedure on the left side. Strength of stimulus, 32 Z-units. February 13, 1924.

PRE-GANGLIONIC STIMULATION			POST-GANGLIONIC STIMULATION		
Series number	Number of observation	Frequency per second	Series number	Number of observation	Frequency per second
I	1	200	III	1	40
	2	140		2	15
	3	120		3	60
	4	80		4	10
	5	60		5	80
	6	40		6	off
	7	15			
	8	off	IV	1	120
II	1	15		2	200
	2	40		3	120
	3	60		4	180
	4	80		5	130
	5	120		6	off
	6	160			
	7	200			
	8	off			
Strength of stimulus, 71 Z-units					
V	Same as series I				
VI	Same as series II		VII	Same as series III	
			VIII	1	80
				2	120
				3	90
				4	110
				5	100
				6	off

In stimulation of the pre-ganglionic fibers a frequency of 40 breaks per second, applied for 1 second, gives a response that is as great as can be obtained with any higher frequency; stimulation of the post-ganglionic fibers with the same strength and frequence causes also a maximal response. Up to a frequency of 120 per second the size of the response stays the same at both points of stimulation. At rates above this, however, a difference appears and increases as the rate rises. If post-ganglionic fibers are stimulated the response *declines* as the rate rises until

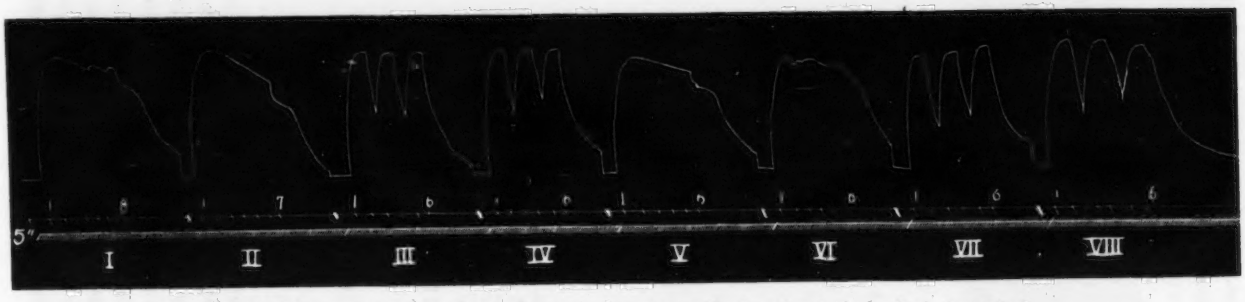

Fig. 4. Difference in the effects of pre- and post-ganglionic stimulation. Data in table 2.

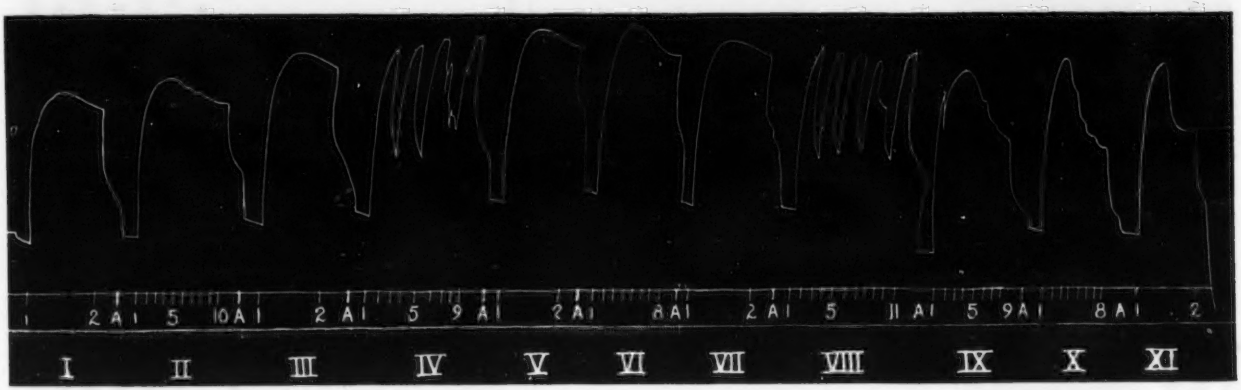

Fig. 5. Difference in the effects of pre- and post-ganglionic stimulation. Data in table 3.

at 180 per second there are only small contractions, and at still higher rates there is no contraction at all. On the other hand, the faster stimulation of the pre-ganglionic fibers produces no change; the same response

TABLE 3 (cf. fig. 5)

Difference of effect on stimulating the pre- and post-ganglionic fibers

Cat 2.8 kgm., 22 cc. urethane by stomach tube, 55 minutes later 3 cc. urethane intraperitoneally. Usual procedure on right side. Strength of stimulus, 42 Z-units. April 1, 1924.

PRE-GANGLIONIC STIMULATION			POST-GANGLIONIC STIMULATION		
Series number	Number of observation	Frequency per second	Series number	Number of observation	Frequency per second
I	1	120	III	1	120
	2	off		2	off
II	1	25	IV	1	60
	2	40		2	15
	3	60		3	80
	4	100		4	10
	5	140		5	100
	6	160		6	160
	7	180		7	150
	8	200		8	140
	9	220		9	off
	10	off			
Strength of stimulus, 65 Z-units					
V	Same as series I		VII	1	80
				2	off
VI	1	200	VIII	1	40
	2	180		2	10
	3	160		3	50
	4	140		4	5
	5	120		5	60
	6	100		6	100
	7	80		7	70
	8	off		8	90
				9	100
				10	75
				11	off

A = drum stopped for 2 minutes.

appears when these fibers are stimulated 180 times per second as when stimulated 120 times.

The disadvantage of this method is that the size of the response may be dependent on the length of the time that the stimulus is applied. In the experiments reported here this was only *estimated* to be one second

by counting. The following procedure is therefore more conclusive. When the rate of stimulation is shifted the primary circuit is not opened, but by quickly changing the resistance in the motor circuit, the frequency is altered while the stimulus is being applied, and the rate is then kept constant at the new frequency. A signal is pressed as soon as the new rate is established and the level of the plateau of contraction indicates whether or not the muscle reacts differently to the new rate. Figure 4 is a typical record of such an experiment and table 2 gives the data.

The record of pre-ganglionic stimulation at rates from 25 to 250 per second shows a smooth plateau of contraction. This is in marked contrast to the response to post-ganglionic stimulation, where reducing the frequency below 40 per second or raising above 160 per second causes a sharp fall in the curve. With stronger stimuli, the critical rates are

TABLE 4

Minimal and maximal critical rates for pre- and post-ganglionic stimulation with different strengths of stimuli

NUMBER OBSERVATIONS	PRE-GANGLIONIC CRITICAL RATE		POST-GANGLIONIC CRITICAL RATE		STRENGTH OF STIMULUS IN Z-UNITS
	Minimum	Maximum	Minimum	Maximum	
4	35	—	40	190	18
17	10	—	25	180	32
8	5	—	20	140	40
13	5	—	20	120	54
16	5	—	15	110	65
9	5	—	10	90	72

lower; the figures given above are for a strength of 32 Z-units; with 45 Z-units a frequency of 140 per second is already critical; while at 72 Z, 90 is the critical point. Cf. table 4.

Different checks were made to ascertain that the second pair of electrodes stimulated post-ganglionic fibers alone. In three experiments a ligature was tied as tightly as possible around the ganglion; in two experiments the ganglion was destroyed with a hot needle; in neither case was there any change in the response to post-ganglionic stimulation. Figure 5 is the record of a complete series (cf. table 3). It shows that, *1*, the shape of the curve, obtained with pre-ganglionic stimulation, is independent of changes in the rate of stimulation; *2*, the shape of curve, obtained with post-ganglionic stimulation, depends on changes in the rate of stimulation. Rates that just fail to evoke maximal responses, when higher frequencies succeed, will hereafter be designated "minimal critical rates;" rates that just fail to evoke maximal responses when lower frequencies succeed will be called "maximal critical rates." In table 4 figures are brought together from different experiments on various critical rates.

b. Influence of different factors on the ganglion. When a warm, diluted nicotine solution is brushed on the ganglion, it is impossible to obtain

TABLE 5 (cf. fig. 6)

Effect of nicotine on the ganglion

Cat 3.2 kgm., 26 cc. urethane by stomach tube. Usual procedure on left side. Strength of stimulus, 32 Z-units. February 18, 1924.

PRE-GANGLIONIC STIMULATION			POST-GANGLIONIC STIMULATION			REMARKS
Series number	Number of observation	Frequency per second	Series number	Number of observation	Frequency per second	
I	1	25	II	1	120	*A*; drum stopped for 2 minutes
	2	40		2	200	
	3	60		3	120	
	4	80		4	180	*B*; drum stopped; 1 drop nicotine 1 per cent applied to the ganglion; drum started again after 4 minutes
	5	120		5	130	
	6	160		6	off	
	7	200				
	8	off	V	Same as series II		
III	1	120	VII	Same as series II		
	2	off				*C*; drum stopped for 2 minutes
			IX	Same as series II		
IV	1	5				*D*; drum stopped for 1 minute
	2	20	XI	Same as series II		
	3	10				
	4	off				*E*; stopped 7 minutes
VI	1	50				
	2	90				*F*; stopped 1 minute
	3	70				
	4	off				*G*; stopped 12 minutes
VIII	1	120				*H*; stopped 1 minute
	2	160				
	3	180				*I*; stopped 5 minutes
	4	220				
	5	180				*J*; stopped 1 minute
	6	200				
	7	190				
	8	off				
X	Same as series I					

any response to stimulation of the pre-ganglionic fibers for several minutes afterwards, while post-ganglionic stimulation within the usual critical rates continues to evoke responses. After some time contraction reappears when the pre-ganglionic fibers are stimulated, and proves to be dependent on the rate at which the stimulus is applied, a low rate causes

contraction, a high one fails. The longer the time after application of the drug, the higher must be the rate that just fails to cause a response. Finally the effect on the ganglion wears off completely so that the membrane responds as before the application of nicotine, and no maximal critical rate can be found for pre-ganglionic stimulation. It is important to note that during an experiment performed in this way the rates of post-ganglionic stimulation, that are found to be critical, do not vary at all, but remain unaffected by the action of nicotine. Figure 6 and table 5 are taken from such an experiment.

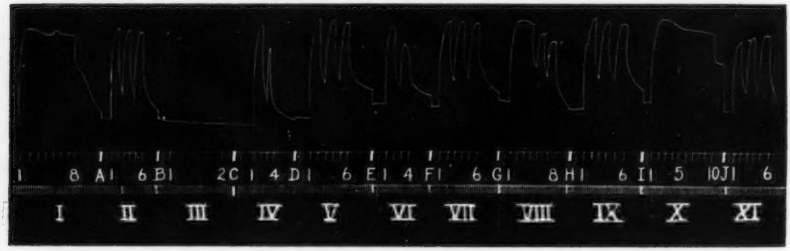

Fig. 6. Effect of nicotine on the ganglion. Data in table 5. Discussion in text.

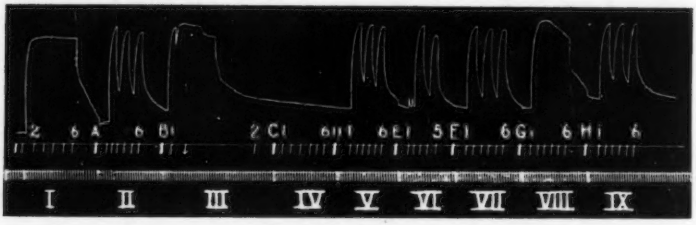

Fig. 7. Effect of cold on the ganglion. Data in table 6. Discussion in text.

The effect of nicotine is not due to a specific action of that drug, for various other substances which might be supposed to cause functional disturbance, make the ganglion respond in the same way. Ethyl chloride was sprayed on the ganglion, cotton soaked in chloroform was placed on it, and ice-cold Ringer solution was applied to it, all with essentially the same result as application of nicotine. When these different agents are allowed to exert their full influence, no response follows pre-ganglionic stimulation. Later, after their removal, when the blocking effect begins to wear off, responses are obtained with low rates but not with high ones. The longer the interval after removal, the higher the critical rate, until finally the response again is independent of the stimulation frequency. The critical rates in post-ganglionic stimulation remain unchanged

throughout. Figure 7 and table 6 give an example of an experiment in which the ganglion was cooled with ice-cold Ringer solution.

Table 7 gives the different maximal rates, found to be critical for pre-ganglionic stimulation, during recovery from disturbance of the function of the ganglion.

TABLE 6 (cf. fig. 7)

Effect of cold water on the ganglion

Cat 2.1 kgm., 16 cc. urethane by stomach tube, 30 minutes later 2 cc. urethane intraperitoneally. Usual procedure on right side. Strength of stimulus, 40 Z-units. March 7, 1924.

PRE-GANGLIONIC STIMULATION			POST-GANGLIONIC STIMULATION			REMARKS
Series number	Number of observation	Frequency per second	Series number	Number of observation	Frequency per second	
I	1	100	II	1	100	*A*; stopped for 1 minute
	2	120		2	200	
	3	140		3	120	
	4	160		4	180	*B*; stopped for 1 minute, then started again, ganglion cooled at arrow
	5	200		5	130	
	6	off		6	off	
III	1	130	V	Same as series II		
	2	off				
			VII	Same as series II		*C*; stopped for 1 minute, still cooling
IV	Same as series I					
			IX	Same as series II		
VI	1	25				
	2	5				*D*; stopped for 1 minute, still cooling
	3	30				
	4	15				
	5	off				
						E; stopped for 1 minute, cooling stopped
VIII	Same as series I					*F*; stopped for 1 minute
						G; stopped for 10 minutes
						H; stopped for 1 minute

c. Effect of cooling the nictitating membrane. In order to cool the nictitating membrane, the contents of the eyeball were removed through a corneal opening, and a cannula placed in the opening directed cold Ringer into the empty eye. The cold solution flowed continuously out along the membrane. Simultaneous stimulating of the pre-ganglionic fibers and starting of the flow of cold water caused the level of the plateau of contraction to fall after a short time. Then, after some irregular contractions, it became impossible to obtain any response to pre-ganglionic stimulation, no matter what rate was employed. On the other hand, it

is easy to evoke contraction when the post-ganglionic fibers are stimulated—the minimal critical rate being higher, the maximal lower, than at the normal temperature.

TABLE 7

Critical rates found on stimulation of the pre-ganglionic fibers while the ganglion was recovering from a disturbing influence

AGENT	NUMBER OF OBSERVATIONS	TIME AFTER REMOVAL	MAXIMAL CRITICAL FREQUENCY (AVERAGE)
		minutes	
Cold Ringer	6	1–2	25
		2–4	80
		4–6	180
Ethyl chloride	2	2–4	15
		4–8	60
		8–12	150
		12–20	200
Chloroform	6	3–6	15
		8–10	60
		12–15	140
		20–25	200
Nicotine	4	6*	15
		15–20	80
		25–30	200

*Time after application.

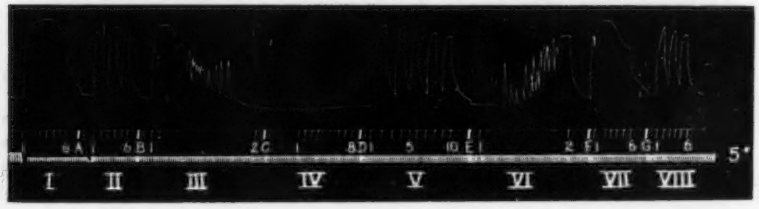

Fig. 8. Effect of cooling the nictitating membrane. Data in table 8. Discussion in text.

If Ringer solution at body temperature is substituted for the cold solution during stimulation of the pre-ganglionic fibers, there is at first no effect, but after a short time irregular contractions are obtained, which merge into the usual smooth plateau. This plateau does not vary thereafter, even though the pre-ganglionic fibers are stimulated at rates ranging from 30 to 260 per second. On stimulating the post-ganglionic fibers

TABLE 8 (cf. fig. 8)

Effect of cooling the nictitating membrane.

Cat 2.5 kgm., 20 cc. urethane by stomach tube. Usual procedure on left side. Strength of stimulus, 45 Z-units. March 26, 1924.

PRE-GANGLIONIC STIMULATION			POST-GANGLIONIC STIMULATION			REMARKS
Series number	Number of observation	Frequency per second	Series number	Number of observation	Frequency per second	
I	1	220	II	1	100	*A*; drum stopped 2 minutes
	2	190		2	200	
	3	160		3	120	
	4	120		4	180	*B*; drum stopped 2 minutes; at arrow nictitating membrane cooled
	5	80		5	130	
	6	off		6	off	
III	1	130	V	1	60	
	2	off		2	100	*C*; stopped 2 minutes; still cooling
				3	50	
				4	90	
IV	1	200		5	40	*D*; stopped 2 minutes; still cooling
	2	180		6	30	
	3	140		7	35	
	4	120		8	80	*E*; cold water changed to warm; drum started after ½ minute
	5	100		9	70	
	6	60		10	off	
	7	15				
	8	off	VIII	Same as series II		
VI	1	120				*F*; stopped 2 minutes
	2	off				*G*; stopped 2 minutes
VII	Same as series I					

TABLE 9

Difference in the effect on pre- and post-ganglionic stimulation when the nictitating membrane is cooled

NUMBER OF OBSERVATIONS	PRE-GANGLIONIC	POST-GANGLIONIC CRITICAL RATE (AVERAGE)		STRENGTH IN Z-UNITS (AVERAGE)
		Minimum	Maximum	
12	no response	40	130	32
2	"	35	120	32
6	"	35	90	45
2	"	30	75	65

we find the usual critical rates. Figure 8 is a record of such an experiment, and table 8 shows the data.

Table 9 gives various critical rates noted when the membrane had been previously cooled in several experiments.

d. Difference in latent period following pre- and post-ganglionic stimulation. In these experiments the latent period was determined by measuring the interval between the moment of closure of the stimulating circuit and the beginning of the contraction, as registered on a rapidly revolving smoked drum, with the time recorded in 0.1 second. This method may perhaps be considered too inaccurate, but since other investigators (9) (14) have attained approximately the same values, I have felt justified in using it.

In different animals the latent period may vary about 0.2 second and, as might be expected, it increases during the course of a long experiment. Since otherwise it remains fairly constant, the method permits decisive observations to be made. If strong stimuli are used, a constant

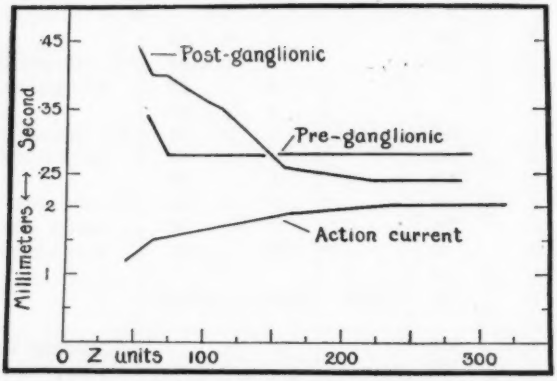

Fig. 9. Relation between strength of stimulus, size of action current and latent period following pre- and post-ganglionic stimulation. Average of 5 experiments.

difference appears between the latent periods, as found in pre- and post-ganglionic stimulation. Stimulation central to the ganglion produces a response after a latent period which is from 0.05 to 0.1 second longer than that following post-ganglionic stimulation.

When the stimulating current is gradually increased from the threshold value, and a constant frequency is maintained that produces maximal responses to both pre- and post-ganglionic stimulation, the following facts were observed: 1, The threshold value for pre-ganglionic stimulation is higher than for post-ganglionic; 2, the latent period following pre-ganglionic stimulation of threshold strength decreases rapidly on the application of stronger stimuli until it attains a constant value, which is not altered by still stronger stimuli; 3, the latent period following post-ganglionic stimulation also decreases as the strength of stimulus is increased from the threshold; it finally reaches a constant value,

but not until the strength of stimulation is much greater than in the case of pre-ganglionic stimulation.

In order to investigate the size of the propagated disturbances, measurements of the action currents were made in the pre-ganglionic fibers, but it proved to be technically impossible to do that with the post-ganglionic fibers.

After recording the latent period, the sympathetic trunk was cut below the superior cervical ganglion, separated from the vagus trunk and mounted in a moist chamber. Stimulating electrodes were applied to the central end and a pair of silver-silver chloride leading-off electrodes (1.5 cm. apart) were placed 3 cm. distal to them. The nerve was crushed between the leads to render the response monophasic. Induction shocks of the same strength as those used in the first part of the experiment were then applied, and the size of the action currents recorded with the string galvanometer.

TABLE 10 (cf. fig. 9)

Relation between strength of stimulus, size of action current and latent period following pre- and post-ganglionic stimulations

NUMBER OF OBSERVATIONS	FIRST STIMULUS FOLLOWED BY MINIMAL LATENT PERIOD (IN Z-UNITS)		FIRST STIMULUS TO SET UP MAXIMAL ACTION CURRENT (IN Z-UNITS)
	Pre-ganglionic	Post-ganglionic	
8	60–75	180–240	170–200
6	30–50	100–125	90–120

The curve which results when the size of the action current is plotted against the strength of the stimulus takes in general the same course as that obtained by Adrian and Forbes (15) from the peripheral nerve-trunk. The size of the excursion fails to increase after a certain point though still stronger stimuli are applied. When the curve reaches this plateau, the response is maximal (cf. fig. 9).

Plotting the strength of stimulus as abscissae, and both the size of the action current and the latent periods for post- and pre-ganglionic stimulation as ordinates (fig. 9) demonstrates that pre-ganglionic stimuli, which are followed by a constant and minimal latent period may not be maximal when judged by the magnitude of the action current they evoke. On the other hand, the first post-ganglionic stimulus with a constant and minimal latent period is almost or exactly the first stimulus to be maximal as determined by the size of the action current. Table 10 gives those relations as found in the different experiments.

DISCUSSION. *a. Inhibition in general.* Before considering more fully the chief subject of this investigation, i.e., the difference between the effects of stimulation on the two sides of the superior cervical ganglion,

the dependence of the response upon the frequency of stimulation of the post-ganglionic fibers and under some conditions also of the pre-ganglionic must be discussed. Examples are well known in the physiology of striated and smooth muscle and of the central nervous system of cases in which a series of stimuli timed at a given interval evoke one response, while stimuli following each other at longer intervals fail; such a condition is termed summation. On the other hand, scattered through the literature are many observations of failure of the end-organ to respond in a usual manner when the interval between the stimuli is less than that producing the summation. Without an attempt at explanation of this phenomenon, it may be called "inhibition."

In 1876, Valentine (16), when stimulating a frog's nerve-muscle preparation with four pairs of electrodes, observed that sometimes stimulation with one pair favors response to stimulation with another pair, and sometimes depresses it. Wedensky (17), (18), (19), in 1885, was the first to investigate this phenomenon more accurately. A nerve-muscle preparation is stimulated until a fall in the level of the plateau of the tetanus indicates fatigue; a decrease of the strength or of the frequency of stimulation evokes a second rise in the contraction curve. Stimulating a fatigued muscle through its nerve with frequent shocks produces a single twitch ("Anfangszuckung") after which the muscle returns to its previous state and remains inactive. Lower stimulation rates will then evoke a steady tetanus. With his telephone method of observing the nature of impulses passing along the nerve, Wedensky found no change during this inhibition; since curare prevents its occurrence, he concluded that its seat must be in the neuro-muscular junction. Later, in a more detailed paper (20), he attempted to give a broader significance to this phenomenon. He found that a tetanus evoked by stimulating a narcotized area of a nerve is inhibited by simultaneous faradic stimulation outside the zone of narcosis. Therefore, the normal impulses arriving at the abnormal area cause inhibition, just as do normal impulses reaching a fatigued neuro-muscular junction. Whether a stimulus evokes a contraction or not depends on the condition of the end-organ, and Wedensky calls the particular state of the irritable tissue, in which the nerve impulse causes inhibition, *parabiosis*.

At the same time (1885) Werigo (21) explained the inhibitory effect of stimulating with two currents simultaneously by assuming mutual interference of the stimulating currents. Six years later tetanic inhibition was reported by Kaiser (22) when the electric stimulus was applied to a nerve which was partially immersed in a glycerin solution. He explained this as an interference of disturbances. Amaya (23) and later Hofmann and Amaya (24) criticized these experiments, because chemical stimulation does not offer constant conditions. They repeated the procedure,

substituting a second electrical stimulus for the chemical stimulation employed by Kaiser: Wedensky's observations were confirmed. They excluded the possibility of electrotonic block, because the direction of either current did not influence the occurrence of the inhibition. They did not offer any explanation for the effect, but they rejected the idea that the apparent inhibition was due to special inhibitory fibers. Groves (25), on the basis of his own experiments, denied that there may ever be inhibition, when a nerve is stimulated either with two currents, or electrically and chemically, at the same time; the reason for his results is probably that he worked with fresh, unfatigued preparations. An explanation quite analogous to Wedensky's was suggested by Hofmann (26); the neuro-muscular junction fatigues and gradually forms a block for the impulses. Froehlich (27, 28) assumed that every impulse, if properly timed, may fall during the refractory period of its predecessor, so that it is unable to reach the muscle, but leaves another refractory period. In his later work Verworn (29) also favored this theory. Woolley (30), without entering into an explanation, reported that inhibition cannot be due to an electrotonic block, established by the more peripheral of two stimulating currents.

In the mean time attention was drawn, especially by Sherrington's work (31), (32), (33), (34), (35), (36), to so-called central or reflex inhibition. We will not discuss here its general significance; Lucas and Forbes (37) have suggested that it may be regarded as a special case of Wedensky's inhibition.

Piotrowsky's (38) work on cray-fish muscle indicated that higher frequencies fail to evoke a response while low ones succeed. Froehlich (39) noted in working with a similar preparation that an excitatory impulse may be rendered inhibitory by increasing its frequency.

Corresponding observations on smooth muscle are rare; partly because it is difficult to differentiate between true inhibition, antagonistic innervation and activity of the antagonistic muscle system. But Langley (40), (41) has observed inhibition proper in bladder and stomach after administration of various drugs. A conclusive observation was made by Mislawsky (42) in work on the smooth muscle of the frog's stomach; he found that a constant current interrupted at suitable intervals provoked a tetanus, and that the level of the contraction fell when the number of interruptions was increased.[1]

[1] In a research, started in 1922, in this laboratory, Dr. H. O. Veach found a definite relationship between frequency and strength of stimulation of the vagus nerve of the cat, and the effects produced on the lower end of the esophagus and the stomach. Increase of strength or of frequency of exciting stimuli evoke inhibition of the Wedensky type. (Science, 1924, lix, 260.)

To Doctor Veach, as well as to other workers in the laboratory I am indebted for some suggestions in regard to this research.

In 1912 Lucas (43) undertook a new investigation of the Wedensky phenomenon. He showed that the absolute refractory period is followed by a relative refractory period, when impulses of subnormal magnitude pass down the nerve. Stimuli, given in the absolute refractory period fail, and do not, as Froehlich supposed, prolong the refractory period, since an additional refractory period only occurs when another propagated disturbance has been set up.

The explanation of Wedensky inhibition given by Lucas is that impulses, traversing the nerve with sufficient frequency, each occur in the relative refractory phase of the predecessor, are therefore subnormal sized, and fail to excite the muscle, except the first one, that passes through full-sized and causes the initial twitch.

Adrian (44) demonstrated in 1913, that increasing the strength of stimulus has the same effect as increasing the frequency. His experiments show that the size of the second disturbance depends only on the interval between the first and the second stimuli and not on the strength of the second stimulus; but each of a series of strong stimuli excites the nerve earlier in the recovery period and is therefore reduced more than weak stimuli that affect the nerve later and suffer less from the change left by their predecessors. Thus strong stimuli excite the nerve early in the recovery period and give rise to weak disturbances; weak stimuli excite the nerve later and therefore give rise to stronger disturbances, and a series of strong stimuli evoke a series of frequent but weak disturbances, unable to affect the muscle. Finally, a series of weak stimuli evoke fewer disturbances, since many fall so early in the recovery period that they cannot excite the nerve; they therefore give rise to a series of less frequent but stronger impulses which are able to excite the muscle.

This explains why, as Wedensky had already found, increasing the strength of stimulus has the same effect as increasing its frequency. Forbes, Ray and Griffith (45) point out that it may be unnecessary to make use of the idea of extra resistance at the neuro-muscular junction, for if the threshold of the muscle is raised by fatigue, subnormal impulses may well fail to excite the muscle.

This explanation allows us to find a common fundamental basis for the different types of inhibition, due to different rates of stimulation which have been described above. The only conditions necessary for the establishment of inhibition are, 1, a recovery period in some part of the conducting path which reduces succeeding impulses to subnormal size when suitably timed, and 2, a resistance more peripheral in the conducting path, which prevents excitation of the irritable substance by the subnormal impulse. The first condition need not be fulfilled in nerve; it may, for instance, be in the conducting mechanism of the muscle itself. The seat of the second condition need not be in the neuro-mus-

cular junction; in case of central inhibition, for instance, the increased resistance (or threshold) may be in one of the synapses in the central part of the conducting path.

b. Inhibition in the special cases of this research. Our own observations can be explained by the principles outlined above. In our experiments inhibition did not always occur with the same tissue as substratum and each must be considered separately.

When working with post-ganglionic fibers, a rate may always be found that is too fast to evoke contraction of the muscle of the nictitating membrane. The stronger the stimulus, the lower is this maximal critical rate; therefore, increasing the strength of stimulus produces the same effect as increasing the rate.

The "Anfangszuckung" which characterizes the Wedensky effect in striated muscle is absent because this twitch is due to the first impulse to reach the muscle, and the muscle of the nictitating membrane is unable to respond to single stimuli—at least of moderate strength—as can be easily proved by stimulating the nerve with single induction shocks.

On the other hand it seems unnecessary to fatigue the preparation in order to obtain the effect; it shows as well in the fresh and unfatigued membrane as it does towards the end of an experiment. In striated muscle fatigue serves to increase resistance or threshold and to prevent the impulse from exciting the contractile substance of the muscle. Even in normal conditions it is more than probable that in smooth muscle there is a resistance which must be overcome before the impulse can excite the muscle. One argument in favor of this hypothesis is the "addition latente"[2] of all smooth muscle, i.e., that the first few of a series of stimuli are without effect, and contraction results only when several have been sent in. More will be said later about the significance of this phenomenon in relation to the latent period. Another argument is that, even under normal conditions, a disturbance appears to be conducted in smooth muscle with a decrement. Most authors (among them Gruetzner (46), Schultz (47), (48), later Alvarez (49)) have assumed without further proof that this is true, and, indeed, the gradual extinguishing of a contraction wave travelling along smooth muscle (intestine, for instance) strongly suggests the existence of such a decrement. These considerations, then, may explain why the Wedensky effect in smooth muscle differs in some ways from inhibition in other irritable tissues.

Stimulation of the pre-ganglionic fibers demonstrates that a broad range of frequencies evoke uniform responses when the ganglion is intact.

[2] Richet in the "Dictionaire de la Physiologie, T. I, p. 147, Paris, 1895, indicates with the term "addition latente" the fact that "des excitations, qui, isolées, paraissent impuissantes, deviennent efficaces quand elles sont repetées."

When its function is disturbed, however, by cold, narcotics, ethyl chloride or nicotine, pre-ganglionic stimulation with strong currents or high frequencies is unsuccessful, although lower rates or weaker currents succeed in evoking contraction. It is clear that under these conditions the seat of the inhibition is not in the smooth muscle. The period of occurrence of inhibition depends on the degree of ganglionic disturbance, and on a stimulation rate which does not prove to be critical for both pre- and post-ganglionic stimulation. The maximal critical frequency observed in post-ganglionic stimulation is the same as when the ganglion is intact, but in pre-ganglionic stimulation the critical rate at the beginning of recovery is much lower than it is later in the experiment, and in general it depends on the intensity of the disturbance of the ganglionic function (cf. table 7). It seems reasonable, therefore, to locate the seat of this inhibition in the ganglion. Here, under normal conditions, there is no resistance sufficient to make the Wedensky effect possible; but during the action of different agents which all tend to impair conduction and raise the threshold for excitation, impulses reaching the ganglion at a suitable frequency will give rise to inhibition.

c. Transformation of frequency of impulses by the peripheral neurones. As already has been pointed out, and as is shown in the protocols, the effects of stimulation at various rates at the two sides of the intact ganglion differ markedly. In every case there is a maximal critical frequency, above which no response can be obtained from the post-ganglionic fibers. Stimulating the pre-ganglionic fibers with the same or a higher rate evokes a contraction which does not differ in size from the response to lower frequencies.

Failure to respond to certain rates signifies that each stimulus falls during recovery from the refractory phase of the preceding impulse, and that there is consequently diminution in size of disturbances, so that they are too small to affect the muscle. Stimuli applied at the same rate to pre-ganglionic fibers do not fail to set up a muscular contraction. We draw the conclusion, therefore, that *impulses at a frequency lower than that of the stimuli pass beyond the ganglion towards the muscle.* Also, since the minimal critical rate for the post-ganglionic fibers is always higher than for the pre-ganglionic fibers, we conclude that in those cases impulses of *a higher frequency than that of the stimuli applied passes over the nerve beyond the ganglion.* Experiments have checked this assumption, i.e., that disturbances set up in the pre-ganglionic fibers are transformed to an optimal frequency after passing through the ganglion. When the ganglion is intact and the membrane cooled, stimulation of fibers central to the ganglion evokes no response at all, no matter what rate is applied; stimulation of the post-ganglionic fibers causes contraction of the cooled muscle. The maximal critical frequency, however, is much lower than

at normal temperature. This observation fits in with the assumption regarding the transformation of the frequency of disturbances set up in pre-ganglionic fibers; for, if the frequency into which the disturbances are transformed is higher than the maximal critical rate for the cooled muscle, it is clear that stimulation of pre-ganglionic fibers will fail to evoke a response. The fact that no rate of pre-ganglionic stimulation evokes contraction proves that the natural frequency of disturbances induced in post-ganglionic fibers is above the maximal critical rate for the cooled membrane. On the other hand, the rate to which pre-ganglionic stimuli are transformed cannot be higher than the maximal critical rate for the membrane at normal temperature.

Therefore, from the data of our experiments we may conclude that *pre-ganglionic stimulation of any frequency above the minimal critical rate sets up impulses which pass beyond the superior cervical ganglion at frequencies between 120 and 160 per second.*

d. Transformation of the strength of stimulus by the peripheral neurones. With post-ganglionic stimulation, the latent period becomes shorter as the strength of stimulus increases, until a constant value is finally reached. Other workers have also observed this fact with various preparations—(Richet (50), Gruetzner (46), Sertoli (51), Bruecke (52) and Ornuma (53)). It may be explained on the same basis as the phenomenon of the "addition latente." According to Nernst's (54) theory, the mechanism of stimulation is a change in the ionic concentration of the irritable cell. Assuming that the propagated disturbance in the nerve fiber acts on the muscle cell as the electric stimulus would act, it is clear that when, in the case of smooth muscle, one impulse does not suffice to establish the required potential difference, a second one, following soon enough after the first, finds still some change in potential left from its predecessor, and adds its effect to that. A third stimulus, sent in at the proper interval, will do the same, and this summation will continue until finally the difference in potential suffices to excite the muscle. This "addition latente" is dependent on the interval between the stimuli only. Since we kept the frequency constant in the experiments on the latent period, the time required for the addition was the same throughout, and the differences in latent periods must, therefore, have another meaning. The latent periods have been shown to vary with the strength of stimulus, and therefore with the number of nerve-fibers excited. When a small number of fibers is excited, they will cause only a few muscle-cells to contract; it is conceivable that a small number of muscle-cells will suffer more from the inertia of the recording system than a large number, since they will be stretched more and it will take a longer time to develop the tension required to lift the lever. A stronger stimulus excites more nerve-fibers and consequently more muscle-cells will act at a time; the tension is

established in a shorter time, and the contraction will start earlier after the beginning of stimulation. When this time becomes constant, the impulses that cause the contraction are maximal. Figure 9 and table 10 show that a constant latent period is obtained with much weaker stimuli applied to pre-ganglionic than to post-ganglionic fibers. Measurement of the size of the action currents demonstrates that disturbances set up in the pre-ganglionic fibers by such stimuli are not maximal. But these submaximal stimuli, when they are sent in to fibers central to the ganglion, are followed by a constant latent period, and we must therefore conclude that the impulses are maximal when they reach the muscle. Since impulses in the post-ganglionic fibers have a minimal latent period only when caused by much stronger stimuli—stimuli that cause in the pre-ganglionic fibers maximal or nearly maximal disturbances—we conclude that *impulses started central to the superior cervical ganglion, no matter of what size, are transformed to impulses of maximal size by passing through the ganglion.* In arriving at this conclusion the assumption has been made that the size of disturbances in post-ganglionic fibers depends only on the number of fibers stimulated, in other words, that post-ganglionic fibers obey the "all-or-none" law. This has not yet been proved directly, but there is no reason here for an exception to the general rule.

e. Theoretical remarks. Our experiments have shown that centrifugal impulses reaching a sympathetic ganglion pass up beyond the ganglion with their frequency and intensity transformed to the optimum. In 1914 Cannon (55), (56) suggested the possibility that the outlying neurones of the autonomic system might transform impulses reaching them from a central source and adapt those impulses to the peculiarities of the tissues innervated. Since the tissues to which he refers—glands and smooth muscle—are not able to respond to sudden, short, frequent impulses as they pass from the central nervous system, this hypothesis is justified. To discover the mechanism of this transformation and its explanation is our next problem.

A priori it might be supposed that transformation takes place within the nerve cell, since that is the only place of actual differentiation in the investigated conducting path. There are still many obscure points in our knowledge of the rhythmic activity of nerve-cells, especially in the mammalian body. The most serious difficulty in the direct measurement of frequency of discharge from a nervous centre is that not one cell but a group always acts at a time, and that the different cells do not start impulses down the nerves at exactly the same moment. Therefore the impulses do not pass through the nerve-fibers simultaneously, and it is impossible to obtain regular excursions in the recording apparatus. Attempts have been made therefore to measure the frequency of response in the organ innervated. Under some circumstances in lower animals

this can be done without difficulty. For instance, Garrey (57), (58) proved that the rhythm of the heart of *Limulus* corresponds closely to the rate of metabolism of the dorsal ganglion. Since Carlson (59), (60) demonstrated the purely neurogenic origin of the heart-beat of *Limulus*, the conclusion may be drawn that the heart rate is an indicator of the rate of discharge of impulses from the ganglion-cells.

Other tissues very suitable for the study of the rhythmic activity in nerve-cells are the special organs of the "electric" fishes *Malopterurus* and *Torpedo*. Garten (61) and several others (62), (63) have recorded with the galvanometer the electrical discharges from these organs, and have found that the frequency corresponds closely to the state of the nerve center and is almost independent of the temperature of the electric organ itself. Each of these responses, therefore, may be regarded as a discharge from the ganglion cells.

Much attention has been paid to the frequency of discharge from the motor cells of the spinal cord, as indicated by the response of skeletal muscle. The different interpretations by Athanasiu (64), Henriques and Lindhard (65) of the electrical response of muscle to stimulation of its nerve-supply need not be discussed here. That tetanic contraction of human skeletal muscle has a rhythmicity of action current of 50 per second is well known (66), but whether this is a rhythm inherent in the muscle, or is the expression of the frequency of nerve impulses, is still not clear. The number of disturbances passing down the phrenic nerve to the diaphragm corresponds to the number of impulses present in the electromyogram (67), (68). For voluntary muscle complicating factors are the proprioceptive impulses which, traveling from the muscle centrally and interfering with centrifugal impulses, increase the number of disturbances (69). In this case there is no direct relation between the frequency of oscillation in the electromyogram and the number of impulses sent down from the motor cells. Buchanan (70) and Forbes and Rappleye (71) have found that the frequency of the electromyogram depends on the temperature of the muscle; the rhythm of a skeletal muscle, therefore, is not a direct indicator of the number of impulses received.

There is strong evidence for the theory that the frequency of discharge from the central nervous system is too high to be followed by the muscle, at least in mammals; but Adrian and Olmsted (72) found that when the *afferent* side of a reflex arc is stimulated with increasingly rapid rates, the muscle follows until 160 to 200 per second are reached; while muscular response follows *efferent* stimulation at a rate as high as 300 per second. It is the central part of the reflex arc, therefore, that seems to impose the lower frequency.

Cooper and Adrian (73), working on frogs, found that when its temperature is raised a muscle follows a higher frequency of stimulation applied to its nerve than is the case at normal temperature. But when stimulated through its reflex arc, the increase of frequency of response at the higher temperature is very slight. Even at normal temperature the muscle seems to follow the frequency of the discharges from the cord — determined by Cooper and Adrian to range between 120 and 150 per second in the frog. Variation in temperature of the cord does not change the number of responses from the muscle, as Buchanan (70) also has observed. The only change is in the shape of the curve, which becomes more and more irregular, with small excursions superposed on the large ones. This is an expression of the fact that the lower the temperature of the cord, the less synchronous is the discharge of the neurones. In contradiction to this Foa (74) describes muscular rhythm as absolutely dependent on the temperature of the discharging center. According to him, only the amplitude of excursion varies with the temperature of the peripheral organ (for frog and toad). This short review shows that there are not many definite data on the rhythmic activity of any nerve cells; there seem to be no data at all for rhythmicity in the nerve cells of the autonomic system.

How we can picture the mechanism which causes the nerve cell to respond with a rhythm of its own—i.e., to transform the rhythm of stimulation which initiates its activity so that impulses of uniform frequency travel along its axone—Forbes and Rappleye have shown in another tissue (71). They explain the fifty-per-second rhythm in the muscle as the resultant of two factors: 1, the rate of recovery of the stimulated tissue, and 2, the size of the stimulus, which in turn depends on the degree of recovery of the conducting path.

In our case also, any explanation must involve the refractory period of the cell, since this phase is at the bottom of all rhythmic activity (75), the frequency of the rhythm evoked in stimulated tissue being the resultant of the two factors: the rate of recovery of the tissue and the strength of the applied stimulus. These factors together determine how soon after one response the tissue recovers enough to react to stimulation of the strength used; in other words, both determine the interval at which disturbances may be set up, and thus the frequency. When the strength of stimulus is increased, the tissue can respond earlier in the recovery phase and the frequency of the response will also increase; but increase of frequency of stimulation without change of the strength may not change the rhythm of responses, because the tissue cannot react any sooner to the stimulus of the given strength than it has already, and stimuli that are sent in before the tissue has sufficiently recovered remain ineffective. This explains how a tissue can respond with a rhythm lower than that of stimulation.

On the other hand, when the ionic concentration of the limiting membrane (according to Nernst's theory, and with the same reservations as made on page 51), as caused by one disturbance, is large enough or returns to equilibrium slowly enough to outlast the duration of the absolute refractory period, it will set up another disturbance during the recovery of the tissue, at the moment when this concentration is adequate to the state of the tissue. In this case the number of responses will be larger than the number of stimuli which evoke them. This explains, without assuming any new property of nerve tissue, how the transformation of frequency which we observed, can take place.

Finally the transformation of strength of stimulus must be considered. Assuming that the "all-or-none-law" holds good for the fibers of the autonomic nervous system, a maximal impulse must be the expression of conduction by all the fibers of the nerve-trunk. If a submaximal impulse in pre-ganglionic fibers sets up a maximal impulse in post-ganglionic fibers, each pre-ganglionic fiber must stimulate several nerve cells, so that more post-ganglionic fibers are involved in conducting the impulse to the periphery. That there is extensive branching in the superior cervical ganglion, and that there are many more nerve cells in the ganglion than medulated nerve fibers supplied to it, has been shown by Ranson and Billingsley (76). Each pre-ganglionic fiber must, therefore, be connected with a number of ganglion cells. An impulse set up in one pre-ganglionic fiber can therefore stimulate several cells, and a magnified disturbance will be transferred to the periphery.

SUMMARY OF RESULTS

1. The experiments described above have shown that the *membrana nictitans* of the cat offers favorable conditions for studying certain peculiarities of the innervation of smooth muscle.

2. Two phenomena similar to Wedensky inhibition are described—one related to the smooth muscle of the nictitating membrane and manifest in the fresh and unfatigued preparation, the other related to the nerve cells of the superior cervical ganglion, and only occurring when the function of the ganglion has been disturbed.

3. Inhibition in the muscle of the nictitating membrane can be obtained by stimulating the post-ganglionic fibers of the superior cervical ganglion with a critical frequency dependent on the strength of stimulus; it is impossible to evoke an inhibition by stimulating the pre-ganglionic fibers even with rates that are 70 per cent higher. On the other hand, maximal contractions may be obtained from pre-ganglionic fibers with rates that are not fast enough to cause a maximal response when applied to post-ganglionic fibers. *The conclusion is drawn, therefore, that impulses of widely varying frequencies, started central to the ganglion, induce there impulses which pass to the end-organ at optimum frequency.*

4. This optimum frequency has been determined as between 120 and 160 per second.

5. In post-ganglionic stimulation the latent period is inversely related to the size of the disturbances—as stimuli become stronger the latent period decreases until with maximal stimuli it is constant; on the other hand, pre-ganglionic stimulation, whether maximal or submaximal, produces a constant latent period. *The conclusion is drawn that disturbances which are started central to the ganglion are transformed to maximal when they pass beyond the ganglion.*

6. Explanation for these phenomena is derived from known properties of irritable tissue.

In carrying out this investigation I have felt indebted to the Nederland-Amerika Fundatie, The Hague, Holland, and to The Rockefeller Foundation, New York, which have enabled me to study in the United States. I wish to express my gratitude to Dr. W. B. Cannon and Dr. Alexander Forbes for the hospitality they have offered me in their laboratories and for their interest and advice. I want to thank Miss Martha Taylor and Miss Anne Hopkins, who helped me to prepare this paper for publication.

BIBLIOGRAPHY

(1) Chauveau, Arloing and Lesbre: Traité d'Anatomie comparée des Animaux domestiques, Paris, 1905, ii, 655.
(2) Owen: On the anatomy of vertebrates. London, 1888, i, 13.
(3) Krauss: Berichte der Ophtalmologische Gesellschaft, 1912, 38.
(4) Froehlich and Loewi: Arch. f. exper. Path. u. Pharm., 1908, lix, 34.
(5) Elliott: Journ. Physiol., 1905, xxxii, 401.
(6) Langley and Anderson: Journ. Physiol.,1892, xiii, 460.
(7) Langley and Dickenson: Journ. Physiol., 1890, xi, 265.
(8) Langley: Journ. Physiol., 1895, xviii, 280.
(9) Lewandowsky: Arch. f. Physiol., 1899, 352.
(10) Martin: The measurements of induction shocks. New York, 1912.
(11) Forbes: This Journal, 1912, lvi, 273.
(12) Martin: Ibid., 1922–1923, lix, 400.
(13) Forbes: Quart. Journ. Exper. Physiol., 1912, v, 149.
(14) Roebroeck: Het ganglion supremum colli nervi sympathici. Utrecht, 1895.
(15) Adrian and Forbes, Journ. Physiol., 1922, lvi, 301.
(16) Valentin: Pflüger's Arch., 1876, xiii, 320.
(17) Wedensky: Ibid., 1885, lxix, 1.
(18) Wedensky: Ibid, 1900, lxxxii, 134.
(19) Wedensky: C. R. Acad. des Sci., Paris, 1890, cxi, 984.
(20) Wedensky: Pflüger's Arch., 1903, c, 1.
(21) Werigo: Ibid., 1885, xxxvi, 519.
(22) Kaiser: Zeitschr. f. Biol., 1891–92, x, 417.
(23) Amaya: Pflüger's Arch., 1902, xci, 413.
(24) Hofmann and Amaya: Ibid., 425.

(25) GROVES: Journ. Physiol., 1893, xiv, 221.
(26) HOFMANN: Pflüger's Arch., 1904, ciii, 291.
(27) FROEHLICH: Zeitschr. f. allg. Physiol., 1904, iii, 468.
(28) FROEHLICH: Ibid., 1907–08, vii, 444.
(29) VERWORN: Arch. f. Physiol., 1900, Suppl., 105.
(30) WOOLLEY: Journ. Physiol., 1907–08, xxxvi, 177.
(31) SHERRINGTON: The integrative action of the nervous system. New York, 1906.
(32) SHERRINGTON: Journ. Physiol., 1894, xviii, 27.
(33) SHERRINGTON: Proc. Royal Soc., London, 1897–98, lxii, 183.
(34) SHERRINGTON: Journ. Physiol., 1899, xxxiii, Suppl.
(35) HERING AND SHERRINGTON: Pflüger's Arch., 1897, lxviii, 222.
(36) SHERRINGTON: Ergebn. d. Physiol., 1905, iv, 797.
(37) FORBES: Physiol. Reviews, 1922, ii, 361.
(38) PIOTROWSKI: Journ. Physiol., 1893, xiv, 163.
(39) FROEHLICH: Zeitschr. f. allg. Physiol., 1907–08, vii, 393.
(40) LANGLEY: Journ. Physiol., 1911–12, xliii, 125.
(41) LANGLEY: Ibid., 1898, xxiii, 407.
(42) MISLAWSKI: Zeitschr. f. allg. Physiol., 1907, vi, 442.
(43) LUCAS: Journ. Physiol., 1911–12, xliii, 46.
(44) ADRIAN: Ibid., 1913, xliv, 384.
(45) FORBES, RAY AND GRIFFITH: This Journal, 1923, lxvi, 553.
(46) GRUETZNER: Ergebn. d. Physiol., 1904, iii, 2, 12.
(47) SCHULTZ: Arch. f. Physiol., 1895, 517.
(48) SCHULTZ: Ibid., 1903, Suppl. 1.
(49) ALVAREZ: The mechanics of the digestive tract. New York, 1922.
(50) RICHET: Journ. de Physiol., 1879, xi, 262.
(51) SERTOLI: Arch. itall. de Biol., 1883, iii, 78.
(52) BRUECKE: Pflüger's Arch., 1910, cxxxiii, 313.
(53) BRUECKE AND ORNUMA: Ibid., 1910, cxxxvi, 502.
(54) NERNST: Goettinger Nachrichte Mathem.-Physik. Klasse, 1899, 104.
(55) CANNON: Amer. Journ. of Psychol., 1914, xxv, 257, footnote.
(56) CANNON: The bodily changes in hunger, pain, fear, and rage. New York, 1915.
(57) GARREY: Journ. Gen. Physiol., 1920, vi, 1, 41.
(58) GARREY: Ibid., 1920, vi, 2, 49.
(59) CARLSON: This Journal, 1904–05, xii, 471.
(60) CARLSON: Ergebn. d. Physiol., 1909, viii, 371.
(61) GARTEN: Zeitschr. f. Biol., 1910, liv, 399.
(62) KOIKE: Ibid., 431.
(63) GOTCH AND BURCH: Proc. Royal Soc., London, 1900, lxv, 434.
(64) ATHANASIU: C. R. Acad. des Sci., Paris, 1922, cxxv, 56.
(65) HENRIQUES AND LINHARD: Pflüger's Arch., 1923, cc, 11.
(66) PIPER: Elektrophysiologie menschlicher Muskeln. Berlin, 1912.
(67) DITTLER: Pflüger's Arch., 1910, cxxx, 533.
(68) GASSER AND NEWCOMER: This Journal, 1921, lvii, 1.
(69) DUSSER DE BARENNE: Skand. Arch. f. Physiol., 1923, xliii, 107.
(70) BUCHANAN: Quart.Journ. Exper. Physiol., 1908, i,225.
(71) FORBES AND RAPPLEYE: This Journal., 1916–17, xlii, 228.
(72) ADRIAN AND OLMSTED: Journ. Physiol., 1922, lvi, 416.
(73) COOPER AND ADRIAN: Journ. Physiol., 1923, lviii, 209.
(74) FOA: Zeitschr. f. allg. Physiol., 1911–12, xiii, 35.
(75) VON UEXKUELL: Ergebn. d. Physiol., 1904, iii, 2, 1.
(76) RANSON AND BILLINGSLEY: Journ. Compar. Neurol., 1918, xxix, 313.

EFFECT OF DOUBLE ADRENALECTOMY ON THE BLOOD COAGULATION TIME IN CATS

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Received for publication June 10, 1924

In the course of some metabolism studies on adrenalectomized cats considerable difficulty was encountered regularly in obtaining blood samples during the last eight to ten hours of the life of each animal owing to the fact that the blood coagulated very rapidly. This rapid coagulation of the blood following double adrenalectomy occurred in every case in a series of 17 cats, and blood samples taken at intervals after the operation showed that the coagulation time became progressively shorter. The details of these tests and their controls are presented below.

Coagulation time was determined by a modification of O. Inchley's method (1). The blood samples were taken on small German silver wire loops, 5 mm. in diameter, made from 0.02 inch wire, and the same wire loops were used throughout the series of experiments. The blood samples, which were obtained from the marginal vein of the ear of the cat as the blood welled freely from a fresh cut, were allowed to coagulate in still room air under as nearly constant conditions as possible as regards humidity, at temperatures ranging from 23 to 29.7°C.

To test the relative accuracy of this modified method the blood coagulation time was determined for 33 normal cats. These readings (see table 1) together with the blood sugar and anal temperature at the time the samples were drawn have been used as checks against the data from the operated series, as both operated and unoperated animals were kept under the same conditions. As the operated animals took little or no food after the operation and were starved for twenty-four hours before the operation, the time of last feeding as well as the size and sex of the animal were noted. From table 1 it may be seen that the coagulation time did not show any marked correlation with these factors, but that coagulation time varies inversely but not proportionally with the temperature of the air in which the blood was allowed to coagulate. This fact has been pointed out previously by Addis (2) for cat's blood and Amendt (3) for a variety of domestic animals. The actual coagulation time for cat's blood as given in table 1, when the

TABLE 1

Blood coagulation time of normal cats

SEX	WEIGHT	ROOM TEMPERATURE	ANAL TEMPERATURE	BLOOD SUGAR	COAGULATION TIME	LAST FEEDING
	kgm.	*°C.*	*°C.*	*per cent*	*minutes*	*hours*
Male	3.7	22.0	38.4	0.086	8.0	18
Male	2.8	22.0			7.0	18
Male	3.3	24.0	38.4	0.086	5.4	18
Male	2.8	25.0	38.2	0.083	4.8	18
Male	2.4	26.0	38.1		4.7	18
Male	3.4	26.0			5.7	18
Male	3.3	26.0	38.2	0.088	4.4	18
Male	2.5	28.0	39.1	0.140	5.8	18
Male	3.1	28.0	38.7	0.084	4.3	18
Male	2.5	29.0	38.3	0.135	2.7	18
Male	2.6	29.7	38.7		2.6	18
Female	1.8	23.5	38.4	0.075	5.4	18
Female	1.6	23.5	38.2	0.082	5.8	18
Female	3.4	23.5	38.4	0.084	6.7	18
Female	2.6	23.5	38.4		3.7	18
Female	3.2	26.0	38.2	0.075	5.0	18
Female	1.8	26.0	37.8	0.152	5.8	18
Female	1.9	26.0	37.4	0.092	3.5	18
Female	1.4	27.0	38.2	0.105	3.0	18
Female	1.4	27.0	38.4	0.100	4.4	18
Female	3.4	27.0	39.1	0.088	4.0	18
Female	3.4	28.0	38.1	0.100	3.4	18
Female	1.8	28.0	38.2		3.8	18
Female	1.8	28.5	38.4	0.080	2.8	18
Female	1.4	28.5	38.3	0.090	2.7	18
Female	3.4	28.5	38.8		2.7	18
Female	3.2	28.0	38.3	0.086	2.6	18
Female	2.6	28.0	39.3	0.090	4.0	18
Male	2.9	27.0			5.6	18
		23.0			7.0	24
		23.0			6.7	50
Male	2.3	27.0			4.7	18
		23.0			8.0	24
		23.0			8.5	50
Male	2.4	27.0			4.4	18
		23.0			5.7	24
		23.0			5.9	50
Male	3.3	28.5			2.4	72
		28.5			*2.65	72
Female	3.1	28.5			2.7	48
		28.5			*3.0	48

*Tested in water vapor.

temperature factor is considered, is approximately the same as that obtained by Cannon and Mendenhall (4) who give an average value of 4.9 minutes for the coagulation time of cat's blood at 25°C. From the last two animals listed in table 1 duplicate samples were run in water vapor following Inchley's method and in air as described above. The samples allowed to coagulate in moist air had a slightly longer coagulation time.

TABLE 2

Effects of ether and operative procedure on blood coagulation time

SEX	WEIGHT	TREATMENT	HOURS AFTER TREATMENT	ROOM TEMPERATURE	ANAL TEMPERATURE	BLOOD SUGAR	COAGULATION TIME
	kgm.			*°C.*	*°C.*	*per cent*	*minutes*
Male	3.1	Ether one hour	Normal	26.0	38.4	0.097	3.8
			2	25.5	38.3	0.096	5.1
			6	25.0	37.7	0.105	6.7
			24	25.0	37.9	0.092	4.6
			144	28.0	38.1	0.094	3.3
Female	3.0	Ether one hour	Normal	26.0	38.1	0.087	4.5
			2	25.5	37.8	0.100	4.6
			6	25.0	38.1	0.120	6.8
			24	25.0	38.3	0.110	3.2
			144	28.0	39.1	0.110	3.5
Female	2.8	Dummy operation	Normal	28.0	38.3	0.085	2.6
			2	26.0	37.6	0.210	4.3
			4	26.0	38.1	0.098	5.9
			18	25.0	38.5	0.095	7.5
			120	28.0	38.5	0.103	3.1
Female	2.6	Dummy operation	Normal	28.0	39.4	0.090	4.0
			2	26.0	39.0	0.280	4.5
			4	26.0	39.1	0.290	3.9
			18	25.5	39.0	0.190	6.5
			120	28.0	39.3	0.096	3.4

Both animals subjected to dummy operation were completely healed in five days. No infection in any case.

The blood sugar determinations were made colorimetrically by the picramic acid method from 0.2 cc. samples, so that including the samples for coagulation the animal rarely lost as much as 0.5 cc. of blood for each reading made.

The double adrenalectomy was performed under ether anesthesia alone, through a single, right, latero-dorsal incision. The operation required approximately 35 minutes and as soon as the animal was sewed up the ether was ventilated out by the administration of an air-carbon dioxide

mixture as described by Henderson, Haggard and Coburn (6). All cats rallied under this treatment in a few minutes and were able to stand in less than a half-hour after the operation.

To check the effect of ether and operative procedure, four animals were run as controls, two of which were merely kept under ether for one hour and the other two were opened while under ether, instruments inserted as in the regular operation, both adrenals exposed and handled, and at the end of 45 minutes the animals were sewed up. Following the ether each of these four animals was given the air-carbon dioxide mixture and treated in every way as the animals subjected to double adrenalectomy. These check experiments are listed in table 2.

In none of these four controls was the coagulation time appreciably lower than the normal at any time during the five or six days following the etherization, but in each animal there was a definite lengthening in the coagulation time between the 6th and the 24th hours. The changes in blood sugar in the two ether controls were slight, but the two animals which were subjected to the dummy operations showed a temporary, but marked hyperglycemia during the first 18 hours, after which they returned to the normal blood sugar level.

In table 3 the detailed data for 5 of the 17 double-adrenalectomized cats are given. All of these animals were under observation at the time they died and the last blood sample was taken immediately after respiration stopped. The blood pressure being very low at this time, it was necessary to cut the throat to obtain blood, so that the last blood sample was taken from the jugular instead of the marginal vein of the ear. The coagulation time of these double-adrenalectomized animals after the operation did not exceed the normal at any time, but the coagulation time became shorter as the death point was approached. Correcting for the lengthening of coagulation time due to a fall in room temperature, there was a progressive shortening of the coagulation time in each case, and an acceleration in the shortening as the animal passed into the last stages of adrenal defficiency. The remaining 12 animals of this series (table 4) also showed a similar shortening of coagulation time following the operation.

This shortening of coagulation time in the double-adrenalectomized animals after operation is in sharp contrast to the normals (table 1), or the controls (table 2) in which there was a definite lengthening of the coagulation time in each of the four cases. Mendenhall (6) working on the effects of various anesthetics on coagulation time in decerebrated cats states that ether hastens the coagulation time and that the effects of ether are wholly through its action on the adrenals, which he demonstrated by the removal of the adrenals. The data from which he draws these conclusions were taken during the first four hours after decebration while the

animal was under the immediate influence of the anesthetic. In the present experiments ether was administered for one hour or less, and then ventilated out by rapid respiration induced by the air-carbon dioxide

TABLE 3

Effects of double adrenalectomy on coagulation time

SEX	WEIGHT	HOURS AFTER OPERATION	ROOM TEMPERATURE	ANAL TEMPERATURE	BLOOD SUGAR	COAGULATION TIME	REMARKS
	kgm.		*°C.*	*°C.*	*per cent*	*minutes*	
Female	2.7	Normal	28.0	38.6	0.103	3.75	
		2	28.0	35.4	0.235	2.60	
		6	29.0	38.3	0.145	2.00	
		12	27.0	38.7	0.102	2.75	
		18	24.0	35.5	0.148	1.15	Animal nervous
		24	29.0	35.4	0.052	0.50	Animal prostrate
		26.3	28.0	34.2	0.050	*	Animal prostrate
		26.5	28.0		0.140	0.8	Just dead
Male	3.1	Normal	27.0	38.8	0.102	4.41	
		2	28.0	36.4	0.172	4.20	
		5	29.0	37.6	0.085	2.15	
		11	28.0	38.8	0.072	2.05	
		18	25.5	36.3	0.083	3.90	
		24	29.0	37.3	0.072	1.35	
		29	28.0	38.0			
		31	29.0	35.3	0.062	2.80	
		36	29.0	35.5	0.054	1.50	
		40.2	29.0		0.125	0.80	Just dead
Female	3.1	Normal	28.5	38.5	0.095	3.00	
		18	28.5	38.0		1.40	
		24.5	28.0	37.8		0.60	Very weak
		34	25.0	34.9		0.50	Dead
Female	2.4	Normal	27.0	37.4	0.088	3.50	
		1.2	27.0	34.3	0.136	2.60	
		15	24.0	34.0	0.048	0.60	Dead
Male	3.3	Normal	28.5	38.3	0.091	3.65	
		18	28.0	38.5		1.60	
		25	27.5	38.6		2.00	
		96	27.5	37.5	0.060	0.80	Very weak
		103	27.5	35.0		0.50	Just dead

*Too rapid to be measured, less than 0.5 minute.

method. The controls so treated did not show reduction in coagulation time at the periods for which the samples were taken, but on the contrary a lengthening of coagulation time. These findings are more nearly com-

parable to the work of Miss Gray (7) on the opossum. She found etherization to increase the coagulation time in both normal and thyroidectomized animals. It seems evident that the shortening of coagulation time found in our double adrenalectomized animals was not the result of etherization.

Shock of operation and hemorrhage have both been considered as factors in this shortening of the coagulation time in these double adrenalectomized animals, but by the operative procedure used the operation was almost bloodless and the controls subjected to dummy operations gave a lengthening instead of a shortening of the coagulation time. Consequently these factors have been rejected.

TABLE 4

Normal and final coagulation time of double adrenalectomized cats

Coagulations taken at room temperature of 25° to 26°C.

SEX	WEIGHT	COAGULATION TIME		PERIOD OF SURVIVAL
		Normal	Fifteen minutes before death	
	kgm.	*minutes*	*minutes*	*hours*
Male	2.45	4.1	0.5	64
Male	2.60	6.7	0.7	40
Male	3.05	6.4	1.3	16.5
Male	3.20	3.5	0.8	32.25
Female	2.80	4.6	1.1	7.25
Female	2.50	4.1	0.5	73
Female	1.75	4.4	0.5	38
Female	2.25	7.6	2.2	36
Female	3.6	3.3	0.4	36
Female	2.5	3.0	0.6	27.25
Female	2.9	5.1	0.9	44
Female	2.7	4.2	1.0	7.5

The figures in tables 2 and 3 suggest a possible correlation between the reduction of the coagulation time and the reduction of blood sugar and body temperature which follows double adrenalectomy. In the operated controls there was a lengthening in coagulation time and also an increase in the blood sugar with little or no change in body temperature, but the greatest lengthening of coagulation time was not synchronous with the most marked hyperglycemia. In the double adrenalectomized animals there was a definite hyperglycemia immediately following the operation and lasting for two or three hours before the onset of the hypoglycemic condition. Similar conditions have been reported by Sakaguchi, Hayashi and Katayama (8) in dogs following double adrenalectomy. During the period of hyperglycemic there was only a slight fall in the coagulation time, but after the hypoglycemia level was reached and the body temperature began to fall more rapidly the acceleration in the coagulation time became

TABLE 5

Barbital and insulin controls

SEX	WRIGHT	TREATMENT	HOURS AFTER TREATMENT	ROOM TEMPERATURE	ANAL TEMPERATURE	BLOOD SUGAR	COAGULATION TIME	REMARKS
	kgm.			*°C.*	*°C.*	*per cent*	*minutes*	
Male	2.5	Barbital*	Normal	24.0	38.1	0.102	8.6	
			2	26.2	35.0	0.080	6.4	Prostrate
			8	25.5	37.0	0.080	6.1	Prostrate
			20	24.5	33.8	0.082	3.8	Prostrate
			44	25.5	36.2	0.100	3.7	Standing
			96	26.0	38.3	0.090	6.9	Standing
Female	1.83	Barbital*	Normal	23.5	38.6	0.096	7.5	
			2	26.2	35.4	0.080	6.6	Prostrate
			8	25.5	37.3	0.068	4.9	Prostrate
			20	24.5	37.3	0.068	7.8	Prostrate
			44	24.0	37.7	0.062	8.1	Prostrate
			96	25.0	38.1	0.100	5.7	Standing
Female	1.85	Barbital*	Normal	23.5	38.1	0.096	7.2	
			2	25.8	34.1	0.095	3.7	Prostrate
			8	25.6	36.4	0.080	8.4	Prostrate
			20	25.0	33.9	0.110	5.8	Prostrate
			44	25.0	34.8	0.080	5.0	Prostrate
			96	25.0	38.1	0.090	5.5	Standing
Female	2.65	Barbital*	Normal	23.5	38.2	0.129	6.4	
			2	25.8	36.6	0.090	8.7	Prostrate
			8	25.5	35.0	0.096	3.3	Prostrate
			20	25.0	33.5	0.100	4.7	Prostrate
			44	25.3	37.2	0.120	5.6	Standing
			96	25.5	39.0	0.080	4.5	Standing
Female	1.82	Insulin†	Normal	23.5	38.6	0.081	6.1	
			2	25.5	37.8	0.056	2.2	Prostrate
			8	25.5	38.5	0.058	2.0	Prostrate
			20	24.2	38.8	0.065	3.0	Prostrate
			44	25.1	39.5	0.080	3.9	Standing
			96	25.0	38.1	0.085	4.7	Standing
Female	2.43	Insulin†	Normal	23.5	38.1	0.082	7.0	
			2	25.5	37.2	0.062	2.6	Prostrate
			8	25.4	37.6	0.054	4.2	Prostrate
			20	24.0	39.3	0.052	4.8	Prostrate
			44	25.0	38.4	0.060	1.7	Standing
			96	25.0	39.2	0.090	9.0	Standing

*Given 200 mgm. per kilo.

†Given 2 rabbit units of Lilly insulin per kilo.

TABLE 5—*Concluded*

SEX	WEIGHT	TREATMENT	HOURS AFTER TREATMENT	ROOM TEMPERATURE	ANAL TEMPERATURE	BLOOD SUGAR	COAGULATION TIME	REMARKS
	kgm.			*°C.*	*°C.*	*per cent*	*minutes*	
Female	1.6	Insulin†	Normal	23.7	38.7	0.120	6.2	
			2	25.5	38.8	0.070	5.7	Prostrate
			8	25.1	38.2	0.076	2.1	Prostrate
			20	24.0	39.3	0.052	4.0	Prostrate
			44	25.0	39.2	0.100	2.0	Standing
			96	25.5	38.4	0.090	6.8	Standing
Female	2.68	Insulin†	Normal	23.9	38.0	0.142	8.5	
			2	25.5	37.4	0.068	6.9	Prostrate
			8	25.1	38.6	0.060	3.7	Prostrate
			20	23.5	38.3	0.078	4.2	Prostrate
			44	24.8	38.2	0.070	1.5	Standing
			96	25.5	38.5	0.085	3.8	Standing

more evident. In order to test the relations between blood sugar, body temperature and coagulation time eight normal cats were divided into two groups, one of which received insulin and the other barbital. The results of these tests are given in table 5.

In the insulin series there was a definite shortening of coagulation and a marked reduction in the blood sugar level between the 2nd and 20th hours after the injection, with little or no change in body temperature. During this period the animals were prostrate and showed the typical muscular tremors which frequently occur during the reduction of blood sugar by insulin. The blood sugar and coagulation time had not returned to normal at the end of the 44th hour following the injection although the animals were standing. The abrupt shortening of coagulation time which was recorded for the 44th hour may have been the result of the excitement of the animal following its efforts to stand while still in a much enfeebled condition. Cannon and Mendenhall (9) state that emotional excitement is the occasion for very rapid clotting. Very little change in body temperature was noted at any time while the animals were under the influence of insulin.

During barbital narcosis the body temperature of the animals of that series was lowered from 1.3 to 4.7°C. The blood sugar was lowered very little as may be seen by a comparison with the normal values in table 1. The coagulation time although lowered approximately 20 per cent between the 8th and 44th hours returns to or above normal as soon as the animals recover from their prostrated condition. Although both insulin and barbital have specific effects in addition to lowering of blood sugar and of body temperature respectively, which render it difficult to make a direct

comparison of those two series with the series of double-adrenalectomized animals, the coagulation time of all three series seems to follow the blood sugar curve rather than the body temperature curve during hypoglycemia.

Cannon and Gray (10) working on cats showed that an increase in dextrose up to 0.4 per cent in the blood did not influence the coagulation time. The lack or a response in coagulation time to this hyperglycemic condition suggests that the parallel fluctuations in coagulation time and blood sugar during the hypoglycemic conditions just described may have been caused by some common factor rather than there being a causative relation between low blood sugar and rapid coagulation time.

A possible explanation of the rapid coagulation of the blood following double adrenalectomy may lie in the presence of excess calcium in the blood after the removal of the adrenal glands. An analysis of the blood of the last of the seventeen cats in this series, from a sample taken just before death, showed a calcium content of 18.7 mgm. per 100 cc. of serum, as compared with 9.1 mgm. for normal cats determined at the same time and 7.34 mgm. for the blood of the cats subjected to the dummy operations. Unfortunately only one determination was made from the adrenalectomized animals so that its real value is uncertain. It is interesting in this connection to point out that Moore and Purinton (11) describe cardiac thrombosis following complete removal of the suprarenal bodies.

SUMMARY

1. The coagulation time in 17 double-adrenalectomized cats was consistently shorter after the operation than before.

2. The coagulation time became progressively shorter in double-adrenalectomized cats as the death point was approached.

3. There was a well-defined parallelism between the curves of blood sugar and coagulation time following the operation after the initial period of hyperglycemia was passed.

4. Control animals indicated that these changes in coagulation time were not the result of ether or operative procedure.

5. Control series of normal animals treated with insulin and barbital respectively showed a similar parallelism between the blood sugar curve and that of the coagulation time when hypoglycemic condition was induced.

BIBLIOGRAPHY

(1) Inchley: Journ. Pharm. Exper. Therap., 1921, xviii, 237.
(2) Addis: Quart. Journ. Exper. Physiol., 1908, i, 305.
(3) Amendt: Pflüger's Arch., 1922, clxxxxvii, 556.
(4) Cannon and Mendenhall: This Journal, 1914, xxxiv, 225.
(5) Henderson, Haggard and Coburn: Journ. Amer. Med. Assoc., 1920, lxxiv, 783.
(6) Mendenhall: This Journal, 1915, xxxviii, 33.
(7) Gray: This Journal, 1924, lxviii, 149.

(8) Sakaguchi, Hayashi and Katayama: Mitt. a. d. med. Fakult. d. k. Univ. z. Tokyo, 1919, xxiii, 33.
(9) Cannon and Mendenhall: This Journal, 1914, xxxiv, 251.
(10) Cannon and Gray: This Journal, 1914, xxxiv, 252.
(11) Moore and Purinton: This Journal, 1901, iv, 51.

ON THE INFLUENCE OF TEMPERATURE ON THE TONUS WAVES OF THE TURTLE AURICLE

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Received for publication June 13, 1924

The tonic oscillations which the auricles of the turtle heart commonly present were first described by Fano (1). In his extensive study of this interesting phenomenon Fano examined the influence of certain physical and chemical agents upon it and repeatedly refers to the effects of temperature, by means of which he tried to separate the two contractile functions of the auricular tissue, i.e., the fundamental or rapid rhythmic beats on the one hand, and the tonus waves on the other.

According to Fano and his co-workers, the action of heat on auricular tissue is depressant and may cause the disappearance of the tonic oscillations; at the same time heat favors the fundamental contractions so that they become more extensive and occur with greater frequence. Cold acts in the opposite sense in that it favors the tonic oscillations and depresses the fundamental contractions (2), (3), (4). An interest in a possible contractile function of the sarcoplasm has led me to repeat the experiments of Fano. The results are presented herewith.

In my experiments I have used the auricles from Pseudemys concinna, Pseudemys elegans, Crysemys marginata, Chelydra serpentina and Graptemys pseudogeographica, always isolated from the body and suspended in a glass chamber by means of two threads tied to them at opposite points. One of these threads was fastened to the tip of an L-shaped glass tube, the vertical portion of which passed through a cork which was pressed into the top of the chamber. The other thread was connected directly to the heart lever through a glass tube set in the cork. Through the cork also there passed a thermometer, the bulb of which reached to the fluid medium in which the auricles were immersed. A small hole at the angle of the L-shaped tube allowed air to flow continuously into the surrounding fluid. The glass chamber above described was itself immersed in a large reservoir filled with water the temperature of which could be varied at will. The spontaneous contractions of the auricles were recorded on a slowly moving drum. A few experiments were performed with the use of Fano's method (fixation of the auricles to a cork by pins), the aim of which is to favor the appearance of the oscillations.

That method, however, did not show great superiority over mine and furthermore required much more delicate handling.

Three different surrounding media were used: the solution used by Fano (NaCl, 0.75 per cent); the common cold-blooded Ringer (NaCl, 7 grams; KCl, 0.30 gram; $CaCl_2$, 0.25 gram; $NaHCO_3$, 0.30 gram; H_2O, to 1000 cc.); and a modification of the latter in which the $NaHCO_3$ was replaced by Na_2HPO_4 to give a final concentration of $1.4—10^{-2}$ gram-molecules and to which sufficient HCl was added to bring the pH to 7.6—a figure which, according to Redfield, is comparable to that in the blood of the turtle. This modification, which Redfield devised, will be referred to hereafter as a "phosphate Ringer."

In my first experiments I used only phosphate Ringer solution as a surrounding medium and I noted that neither heat nor cold acts in the manner described by Fano and his co-workers, but rather in the opposite sense (fig. 1). Reference to this figure makes clear that warming the solution causes the tonus waves to occur more frequently and accelerates the rate of the rapid fundamental contractions. On the other hand, cooling depresses the tonic oscillations and may cause them to disappear; at the same time the intervals between the fundamental contractions are lengthened until they also disappear. In ten experiments, using phosphate Ringer, I have not failed to see these phenomena occur.

The favorable action of heat on the tonus waves is demonstrated not only by causing their more frequent occurrence, but also by inducing their appearance in auricular tissue which has not previously manifested them (fig. 2). Again, the application of heat may cause the reappearance of tonic oscillations which have been depressed or abolished by the action of atropine.

After these results had been observed, it seemed possible that the difference between them and those reported by Fano was due to difference in the kinds of turtles used. At that time I had used only Pseudemys concinna—he used Emys europaea. I then tested the other forms mentioned above and obtained the same results as with P. concinna. It seemed improbable, therefore, that the difference between Fano's results and mine was due to specific differences in the turtles. I next tried changing the medium surrounding the pulsating tissue and found that the results which I had obtained using phosphate Ringer were not obtained with salt solution (NaCl, 0.75 per cent). With salt solution I only once observed a positive effect of heat on the tonic oscillations (fig. 3, *A*); later replacement of the salt solution by phosphate Ringer, however, induced waves which were much more pronounced (fig. 3, *B*). In all other instances in which the tissue was immersed in sodium chloride solution, increase of temperature had a negative effect, such as Fano described. In the majority of my experiments I controlled the negative effect of heat found

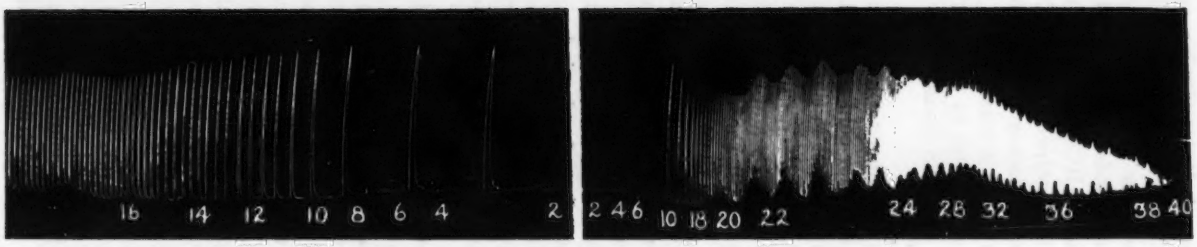

Fig. 1. Effect of cooling and heating upon the tonic oscillations of the auricular tissue of the turtle's heart. The numbers below the record represent degrees Centigrade.

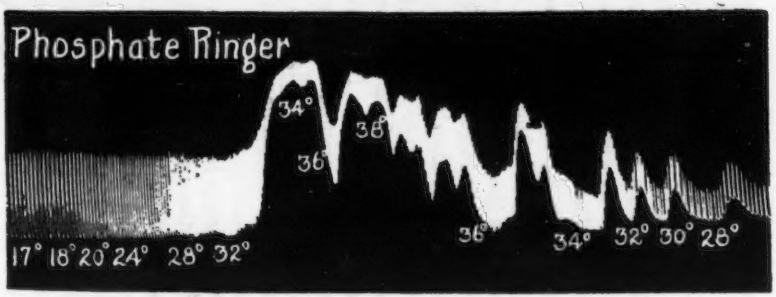

Fig. 2. Effect of heating upon the tonus of the auricular tissue of the turtle's heart. The numbers below the record represent degrees Centigrade.

when the preparation was immersed in salt solution by changing to phosphate Ringer and observing the positive effect.

Rarely, cold seems to have a positive effect, since it causes the tonic oscillations to be prolonged. Examination of the tracings published by Fano gives the impression that this action of cold was the reason for his inference that it has a positive effect. It is not possible to be sure of this, however, because he did not publish the normal shape of the tonic oscillations before the cooling. In any case the prolonging of the natural

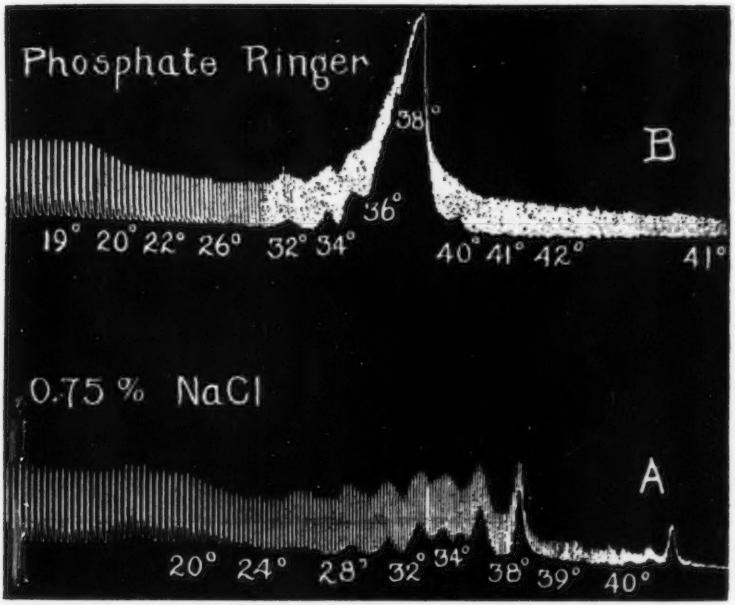

Fig. 3. Effect of heating upon the tonus of the auricular tissue of the turtle's heart immersed in different surrounding media. The numbers below the records indicate degrees Centigrade.

spontaneous waves by cold is quite different from the positive initiation of a series of waves by heat (cf. figs. 1 and 2).

Fano has explained the tonic oscillations in auricular tissue as a consequence of a preponderance of the anabolic over the catabolic process. This idea he supported by assuming that heat has essentially a catabolic effect, and therefore is antagonistic to the tonic oscillations. The experiments presented above do not lend support to his view. There is no reason to suppose that tonic oscillations, which are also muscular contractions, represent phases of restoration, as Fano's view would imply.

I wish to acknowledge my indebtedness to Doctors Cannon and Redfield for their helpful criticisms and valuable suggestions during the performance of this work.

BIBLIOGRAPHY

(1) FANO: Beiträge zur Physiologie Carl Ludwig gewidmet, Leipzig, 1887, 287.
(2) FANO AND SCIOLLA: Arch. Ital. de Biol., 1888, ix, 61.
(3) FANO AND FAYOD: Arch. Ital. de Biol., 1888, ix, 143.
(4) FANO AND BADANO: Arch. Ital. de Biol., 1900, xxxiv, 301.

DAY TO DAY VARIATIONS IN BASAL METABOLISM OF WOMEN

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Although in a general way it is known that the basal metabolism of an individual varies somewhat from day to day, there are very few records of daily observations running over a long period. The best series of the sort is probably that reported by Kunde, (5) with daily observations on one subject throughout a year. Several such series are needed in order to determine the causes and the extent of the variations to be expected in basal metabolic rate. In our experiments, observations have been made upon two subjects almost daily over a period of four months.

Method. The observations were made, between 9 and 11 a.m., on subjects who had fasted for twelve to fifteen hours. The subject lay quietly on a couch for about thirty minutes, then took the mouth-piece for fifteen minutes before the experiment which lasted five minutes. Outdoor air was breathed and the expired air was led to a wet gas meter through an air-mixer from which a continuous sample was taken (16). Analyses were made with the Haldane apparatus. Vacuum sweeper tubing served very well for the long connections with gas-mask tubing for the short turns. For the mouth-piece, we found the Krogh shape by far the most comfortable. Valves of the rubber flutter type were used. This method is accurate to within ± 2 cc. O_2 per minute.

Results. The experiments were run almost daily from early in February until the end of May, giving 96 observations on M. E. C. and 80 on R. H. The complete data are given in tables 5 and 6 and in figures 1 and 2. The material is analyzed in the following briefer tables.

As table 1 shows, the average basal metabolism of both subjects is 5 to 8 per cent below standard. This may be due to the warm climate, for during the colder weather both subjects approached more nearly to the normal (table 2). There was a difference of 5 per cent for both subjects between the averages for warm and cold weather. M. E. C.'s metabolism during the cold days in New Orleans was very close to the level observed a year ago in Stockholm. Whether this difference in metabolism with change in temperature is due to changes in muscular tone associated with muscular activity or solely to diet, we do not know. Differences of even greater magnitude have been reported from the tropics (7).

Average values for the whole series are shown in table 3. In calculating these variations we have omitted experiments in which the subject was

known to be disturbed. The maximum variations in oxygen consumption corresponds closely to that found by Kunde (+13 per cent) and −8.3 per cent). The average variation for M. E. C. was ±4 per cent, somewhat less than for the more nervous subject R. H. (+5 and −6.3 per cent).

TABLE 1

Standards of basal metabolism (cc. O_2 per minute)

SUBJECT	AGE	CENTIMETERS	KILOGRAM	SQUARE METER	OBSERVED	HARRIS BENEDICT	AUB-MEANS DU BOIS	DU BOIS
M. E. C.	35	156	60	1.59	179	195	195	201
R. H.	27	163	50	1.52	181	191	189	195

TABLE 2

Temperature and basal metabolism (cc. O_2 per minute)

TEMPERATURE	M.E.C.	R.H.
°C.		
20–30	176 (41 days)	174 (25 days)
3–10	186 (20 days)	185 (17 days)
0–10	191 (38 days)	

TABLE 3

Amount of variation

		PULSE	VENTILATION	cc. O_2	R.Q.	PER CENT CO_2	PER CENT DEVIATION OF O_2
Average:							
M. E. C.		68.5	3.77	179	0.777	3.69	
R. H.		71.3	4.71	181	0.805	3.05	
Maximum deviation:							
M. E. C.	Positive	1.5	0.47	23	0.188	0.53	+12.8
	Negative	3.5	0.41	17	0.081	0.41	−9.5
R. H.	Positive	9.0	0.73	16	0.23	0.68	+8.8
	Negative	5.0	0.75	26	0.105	0.25	−14.4
Average deviation:							
M. E. C.	Positive	1.7	0.18	7.7	0.056	0.155	+4.3
	Negative	1.8	0.16	7.9	0.033	0.159	−4.4
R. H.	Positive	2.7	0.23	9.1	0.052	0.145	+5.0
	Negative	1.6	0.30	11.4	0.050	0.138	−6.3

In R.H. the variation in pulse and ventilation, though small, is considerably greater than in M. E. C. It is interesting to note the difference in R. Q. and percentage of CO_2 of the subjects, percentage of CO_2 being consistently higher in M. E. C., and R. Q. slightly lower. In every way, these figures reflect the difference in nervous condition of the subjects.

Variations from the average basal metabolism are sometimes impossible to explain, but in a considerable number of cases, we have been able to correlate them with definite conditions of the experiment. Among the accidental variations we may include any condition which interferes with the relaxation of the subject. Harsh or sudden noises, even though not loud, often increase metabolism by 10 per cent or more (M. E. C. 20/3, 5/4; R. H. 7/2, 10/2, 29/3, 8/4). M. E. C. generally recovers from such disturbances in about five minutes, while R. H. often requires fifteen to twenty minutes. The operator is often quite unconscious of noises which seriously disturb the subject. Slight chilling may also increase metabolism, although we observed no effect until we felt slightly shivery (R. H. 20/2 and 21/2). The effect wears off in about ten minutes. Even in a room of 20°C. it is well to use a light blanket to guard against chilling. On two very hot days a high metabolism was also observed, due apparently to restlessness (M. E. C. 24/4 and 24/5). Anxiety, excitement, overfatigue or indeed anything tending to interfere with relaxation result in increased metabolism, even though the subject seems to be lying perfectly quiet throughout the resting period as well as during the experiment (M. E. C. 29/3, 12/5, 19/5 and R. H. 19/2., 11/4, 12/4. 22/5). Fatigue, if not associated with nervousness, sometimes reduces metabolism (M. E. C. 7/4 and R. H. 29/2). Slight digestive upsets and infectious colds cause an increased metabolism at first, followed by a drop below normal after the fever (M. E. C. 7/2, 9/2; R. H. 15/5 to 19/5). Discomfort or pain may raise the level considerably (M. E. C. 16/4; R. H. 16/2, 20/3).

Correlation between basal metabolism and the menstrual cycle appears to be a moot point. Several authors state that they have found no evidence of rhythm (13), (4), (2), (12), (1), and in two recent surveys of literature (7), (2), a similar stand is taken. In the experimental papers just cited the method is open to objection; the observations were generally made over a short period and at irregular intervals, scarcely enough to establish the normal average, or to catch brief but significant variations that might occur. Even in these reports, however, there is some evidence of a premenstrual rise and a menstrual drop in metabolism (Collettt and Liljestrand (3) on analysis of the work of Zuntz and Wakeham (11) of Blunt and Dye.) Wakeham divided the cycle into five-day periods, beginning with the first day of menstruation and plots the averages. He finds remarkable agreement between his observations and those of Blunt and Dye in the occurrence of a variable premenstrual rise followed by a menstrual drop of 4 to 8 per cent. A similar rhythm is reported by Snell, Ford and Rowntree (10) and by Rowe and Eakin (9). Kunde states that although the relation is not constant "the tendency seems to be toward a lowering of basal metabolism during the first four days of menstruation." The fact that variations of equal magnitude may occur at random does

not mean that a regularly recurring premenstrual rise or menstrual fall is without significance, although this seems to be the gist of current criticism. In our own experiments, the graphs show *a definite tendency toward a rise before each period and a sharp drop on the first or second day of menstruation*, unless interfered with by pain. The drop may appear just before the period or it may continue for some days after. Generally, recovery of the normal level is slow if the subject is tired or out of condition. There is frequently, but not always, an intermenstrual minimum. A tendency toward rhythmic variation is also to be observed in ventilation, percentage of CO_2 and respiratory quotient. We could find no evidence of a regular variation in pulse rate such as that reported by Moore and Cooper (8), perhaps because our observations were made in a thoroughly relaxed state.

TABLE 4

Final averages in five-day periods

	−10	−5	M_{1-2}	+5	+10	+15	+20	AVERAGE
cc. oxygen per minute:								
M. E. C., 1923, Stockholm	193	191	184	189	192	185	197	189
M. E. C., 1924, New Orleans	180	175	173	176	179	177	180	179
R. H., 1924, New Orleans	181	179	171	181	176	176	176	181
M. K., 1921–22, Chicago:								
Before fast	198	210	194	197	196	209	209	199
After fast	214	216	206	212	211	212	220	213
Liters ventilation per minute:								
M. E. C., 1923	4.6	4.4	4.3	4.3	4.3	4.5	4.7	4.3
M. E. C., 1924	3.9	3.9	3.6	3.8	3.6	3.7	3.7	3.8
R. H., 1924	4.6	5.0	4.8	4.8	4.5	4.5	4.6	4.7
Percentage CO_2 expired air:								
M. E. C., 1923	3.31	3.56	3.51	3.59	3.66	3.47	3.40	
M. E. C., 1924	3.58	3.65	3.87	3.79	3.73	3.74	3.69	3.69
R. H., 1924	3.05	3.07	2.93	3.02	3.09	3.03	3.04	3.05

In order to eliminate confusing chance variations, we have followed Wakeham's scheme and have made averages for each cycle divided into five-day periods. The final averages for all cycles treated in this manner appear in table 4 and figure 1. Differences of 2 per cent and less are not significant. We have analyzed, in addition to our own data of this year, the observations of a year ago on M. E. C. and the complete series reported by Kunde. Graphs of each cycle made separately agree very well with the graph of the final average. In all three subjects there is a fall of about 5 per cent below the average level just before or during the period, followed at once by a return to normal or above it. The return to normal is more rapid when the subject is in good health and muscularly active. A premenstrual rise frequently occurs lasting a day or so (M. E. C.) or

longer (M. K.). The highest level in M. E. C. and M. K. occurs about twenty days after the first day of the period and in R. H. at five, twenty-five and thirty days after. Intermenstrual minimum is more definite and lasting in R. H. than in either of the other subjects. The reason for these

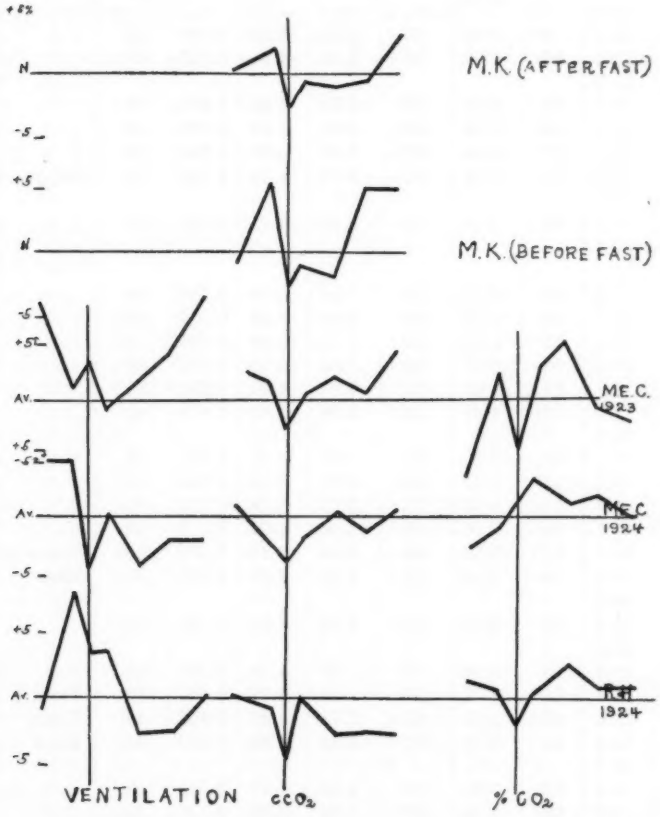


Fig. 1. Average values for all cycles taken in five-day periods.

rhythmic fluctuations is not clear. The permenstrual rise may be partly due to nervousness and the drop to lessened tension, or it may be associated with metabolic variations directly due to thyroid activity. The post-menstrual rise and the intermenstrual minimum can scarcely be explained in this way.

TABLE 5

Basal metabolism—M. E. C.

Rate per minute

DATE	TEMPERATURE	PULSE	LITERS VENTILATION	CC. O_2	PER CENT CO_2	PER CENT O_2	R.Q.	MINUTES REST	NOTES
1/2	15.6	70	3.82	175	3.42	4.58	0.747	47	
2/2	13.3	66	4.02	198	3.55	4.91	0.723	48	
3/2	15.6								
4/2	16.1	65	3.75	180	3.74	4.81	0.778	45	
5/2	3.4	70	3.78	186	3.63	4.91	0.740	47	
6/2	3.3	72	3.64	189	3.87	5.20	0.744	45	
7/2	9.5	71	3.58	175	3.71	4.88	0.760	53	Grippe p.m.
8/2	7.2								
9/2	8.3	69	3.49	166	4.22	4.75	0.888	50	
10/2	14.4								
18/2	17.2	66	3.50	195	3.94	5.96	0.710	60	
19/2	16.7	68	3.60	189	3.76	5.25	0.716	50	
20/2	8.3	71	3.60	189	3.81	5.25	0.725	55	
21/2	11.5	66	3.75	180	3.54	4.80	0.740	40	
22/2	6.7	68	3.66	176	3.60	4.80	0.750	50	
23/2	15.0	72	3.90	172	3.66	4.42	0.828	45	
24/2	13.9								
25/2	12.2	68	3.90	202	3.60	5.17	0.696	50	
26/2	9.5	70	3.45	189	3.86	5.48	0.705	55	
27/2	5.6	70	3.87	184	3.67	4.76	0.771	45	
28/2	9.5	70	3.96	188	3.39	4.74	0.715	60	
29/2	12.8	68	3.82	198	3.61	5.19	0.675	60	Warm bath
1/3	13.9	70	3.50	168	3.66	4.80	0.762	45	M_1
2/3	13.3								
3/3	13.3	68	3.62	176	3.88	4.87	0.796	70	
4/3	17.8								
5/3	19.4	70	3.58	172	3.96	4.79	0.826	55	
6/3	16.1	68	3.72	175	3.98	4.71	0.845	40	Tired
7/3	15.6	69	3.76	166	3.74	4.40	0.850	45	Tired
8/3	14.4	69	3.77	177	3.44	4.70	0.732	45	Tired
9/3	16.1								
10/3	4.4	70	3.80	181	3.60	4.77	0.755	55	
11/3	4.6	68	3.75	187	3.76	4.99	0.755	50	
12/3	7.6	70	3.75	189	3.70	5.04	0.735	50	
13/3	7.8	70	3.60	186	3.83	5.15	0.725	50	
14/3	4.4	70	3.69	187	3.76	5.06	0.742	50	
14/3	4.4	68	3.53	189	3.86	5.35	0.710	45	
15/3	7.2	70	3.96	189	3.61	4.78	0.755	45	
16/3	10.0								
17/3	15.0								

Ventilation reduced to 0°C. 760 mm.
Temperature Centigrade degrees at 9 a.m. (Weather Bureau).

TABLE 5—*Continued*

DATE	TEMPERATURE	PULSE	LITERS VENTILATION	cc. O_2	PER CENT CO_2	PER CENT O_2	R.Q.	MINUTES REST	NOTES
18/3	13.9	68	3.94	183	3.56	4.64	0.766	45	
19/3	12.2	71	3.86	179	3.54	4.64	0.762	42	
20/3	9.5	69	4.11	193	3.44	4.70	0.731	60	Noise
21/3	8.3	70	4.15	169	3.44	4.08	0.844	50	
22/3	9.5	70	3.95	185	3.51	4.67	0.751	45	
23/3	18.3								
24/3	15.6	68	3.92	184	3.72	4.69	0.793	65	
25/3	15.6	70	3.58	165	3.86	4.60	0.838	40	
25/3	15.6	70	3.84	172	3.74	4.47	0.837	53	
26/3	17.8	70	3.81	166	3.90	4.35	0.895	45	M_2
27/3	20.5	69	3.68	166	4.09	4.51	0.905	45	
28/3	24.4								
29/3	24.4	68	3.77	176	3.77	4.68	0.778	40	Excited
30/3	21.7								
31/3	21.1	70	3.55	186	3.92	5.17	0.761	45	
1/4	8.3	67	3.85	186	3.44	4.83	0.712	40	
2/4	10.6	66	3.58	184	3.75	5.13	0.731	45	
3/4	15.6	68	3.56	180	3.76	5.06	0.743	50	
4/4	16.7	66	3.44	182	3.44	5.28	0.762	40	
4/4	16.7	66	3.36	176	4.02	5.15	0.782	45	
5/4	17.8	68	3.59	181	3.94	5.05	0.780	45	Noise
6/4	21.1								
7/4	20.5	66	3.66	167	3.88	4.56	0.850	45	
8/4	18.9	69	3.78	185	3.74	4.89	0.764	40	
8/4	18.9	69	3.98	181	3.60	4.53	0.796	55	
9/4	21.7	68	3.74	171	3.52	4.58	0.769	40	
10/4	19.4	66	3.76	176	3.71	4.67	0.794	40	
11/4	14.4	69	4.16	195	3.39	4.69	0.723	40	Restless
11/4		69	3.77	167	3.53	4.43	0.796	45	
12/4	18.3	70	3.92	182	3.43	4.64	0.740	45	Noise
13/4	20.0								
14/4	21.1	68	3.96	189	3.65	4.77	0.765	40	
15/4	20.0	69	3.82	170	3.73	4.45	0.838	45	
16/4	20.0	70	3.85	162	3.88	4.22	0.924	50	
17/4	23.3	70	4.04	163	3.90	4.04	0.965	45	
18/4	17.8								
19/4	18.3	67	3.73	185	3.82	4.96	0.770	50	Restless
20/4	20.0								
21/4	21.1								
22/4	22.8	68	3.56	179	4.09	5.04	0.805	60	M_1 (lecture)
23/4	24.4	70	3.96	184	4.22	4.66	0.905	45	
24/4	23.9	69	3.76	172	4.03	4.57	0.881	47	
25/4	24.4	68	3.60	170	4.19	4.61	0.908	70	
26/4	24.4	68	3.84	187	3.46	4.88	0.710	45	Noise
27/4	22.8								
28/4	25.6	66	3.52	182	3.72	5.16	0.721	80	Lecture

TABLE 5—*Concluded*

DATE	TEMPERATURE	PULSE	LITERS VENTILATION	CC. O_2	PER CENT CO_2	PER CENT O_2	R.Q.	MINUTES REST	NOTES
29/4	25.0	66	3.62	173	3.58	4.77	0.751	80	
30/4	18.9	66	3.44	167	3.67	4.86	0.755	45	
1/5	15.0	68	3.68	179	3.76	4.88	0.770	55	Noise
2/5	22.2	69	3.66	178	3.64	4.88	0.747	40	
3/5	22.8	69	3.88	178	3.40	4.60	0.740	55	
4/5	23.3								
5/5	23.9								
6/5	24.4	70	3.84	188	3.72	4.90	0.760	45	
7/5	22.9	66	3.76	174	3.66	4.63	0.791	45	
8/5	20.0	68	4.00	162	3.44	4.05	0.850	50	
9/5	22.2	68	4.05	186	3.56	4.59	0.775	45	
10/5	18.9	70	3.95	180	3.49	4.58	0.761	50	
11/5	15.1								
12/5	20.0	68	4.17	193	3.55	4.64	0.766	65	Excited
13/5	22.2	68	4.10	182	3.48	4.44	0.784	60	
14/5	23.9	69	4.20	177	3.28	4.20	0.781	40	
15/5	19.4	69	4.24	178	3.36	4.22	0.796	45	
16/5	20.5	66	3.92	170	3.44	4.35	0.791	50	
17/5	23.9	68	4.00	185	3.56	4.61	0.771	50	
18/5	23.3	66	3.92	181	3.40	4.61	0.726	55	M_1
19/5	26.2	68	3.60	180	3.68	5.01	0.734	75	Excited
19/5		68	3.57	178	3.74	4.99	0.750	95	
20/5	27.2	69	3.92	171	3.42	4.36	0.784	45	
21/5	20.0	69	3.87	169	3.82	4.37	0.874	60	
22/5	21.7	68	3.57	166	3.67	4.66	0.787	60	
23/5	23.3								
24/5	26.2	67	4.00	195	3.68	4.89	0.752	50	
24/5		66	3.54	176	3.82	4.98	0.768	50	
25/5	27.2								
26/5	27.2	66	3.49	175	3.87	5.03	0.770	45	
27/5	27.8								
28/5	27.8	66	3.60	180	3.61	4.99	0.724	55	
29/5	27.8								
30/5	28.9	66	3.82	183	3.74	4.81	0.780	50	

Ventilation follows the general course of the O_2 curve rather closely for M. E. C. but in R. H. is unduly high for five days before and after a period. This irregularity is probably due to the highly nervous condition of the subjects and to acute pelvic discomfort or pain, a condition reflected in the low CO_2 of the first or second day. In M. E. C. the percentage of CO_2 is lowest ten days before the period, while the highest point occurs during the period and sometimes persists beyond it. A condition which we are unable to explain is the high ventilation of the five days before as compared with the five days after a period when the percentage of CO_2 and cubic centimeters O_2 per minute are nearly identical.

TABLE 6

Basal metabolism—H. R. H.

Rate per minute

DATE	TEMPERATURE	PULSE	LITERS VENTILATION	cc. O_2	PER CENT CO_2	PER CENT O_2	R.Q.	MINUTES REST	NOTES
6/2	3.3	80	3.96	172	3.73	4.35	0.86	75	
7/2	9.5	71	4.50	181	3.12	4.03	0.774	42	Noise
8/2	7.2								
9/2	8.3	72	4.06	176	3.15	4.34	0.730	45	
10/2	14.4	70	4.92	197	3.15	4.01	0.750	55	Noise
11/2	14.4								
12/2	14.4	74	4.90	172	3.37	3.52	0.957	45	
13/2	12.2								
14/2	13.3	72	4.86	187	3.23	3.85	0.840	60	
15/2	15.0	71	4.70	187	3.13	3.99	0.784	45	
16/2	14.4	74	4.84	203	3.93	4.20	0.697	45	M_1—Pain
17/2	18.3	73	4.78	186	3.05	3.89	0.784	55	
18/2	17.2	70	4.85	189	3.17	3.89	0.815	45	
19/2	16.7	72	4.73	195	3.19	4.12	0.774	42	Excited
20/2	8.2	71	4.62	186	3.16	4.03	0.785	42	Chilly
21/2	7.2	70	4.93	187	3.04	3.80	0.800	42	Chilly
22/2	6.7	71	4.33	176	3.06	4.06	0.754	47	
23/2	15.0	73	4.56	170	3.10	3.73	0.830	40	
24/2	13.9								
25/2	12.2	69	4.13	191	3.22	4.62	0.700	40	
26/2	9.5	70	4.31	194	3.10	4.50	0.690	45	
27/2	5.6	71	4.41	181	3.04	4.10	0.742	40	
28/2	9.5	72	4.40	175	3.02	3.98	0.758	55	
29/2	12.8	73	4.46	165	3.03	3.70	0.822	45	Tired
1/3	13.9	66	4.47	174	3.14	3.90	0.805	40	
2/3	13.3								
3/3	13.3	70	4.75	185	3.18	3.91	0.814	45	
4/3	17.8								
5/3	19.4	69	4.45	168	3.12	3.80	0.820	40	Tired
6/3	16.1	69	4.73	172	3.16	3.64	0.868	40	
7/3	15.3	71	4.55	180	3.22	3.96	0.814	60	
8/3	14.4	73	5.00	193	3.10	3.87	0.800	90	Noise
9/3	16.1								
10/3	4.4	72	4.87	190	3.21	3.91	0.821	40	
11/3	5.6	72	4.81	194	3.14	4.04	0.777	40	
12/3	7.2	74	4.73	186	2.98	3.94	0.756	45	
13/3	7.8								
14/3	4.4	75	4.62	177	3.02	3.84	0.787	45	
15/3	6.2	72	5.14	197	2.87	3.83	0.749	40	
16/3	10.0	74	5.11	187	2.88	3.65	0.789	40	
17/3	15.0	74	5.12	173	2.82	3.39	0.831	45	
18/3	13.9	72	4.36	157	2.89	3.61	0.800	40	M_1

Ventilation reduced to 0°C. 760 mm.
Temperature Centigrade at 9 a.m. (Weather Bureau)

TABLE 6—*Continued*

DATE	TEMPERATURE	PULSE	LITERS VENTILATION	CC. O_2	PER CENT CO_2	PER CENT O_2	R.Q.	MINUTES REST	NOTES
19/3	12.2	72	4.61	177	2.88	3.83	0.750	40	
20/3	9.5	72	4.77	187	3.02	3.93	0.768	40	Poor sleep
21/3	8.3	73	4.74	186	2.88	3.93	0.733	60	
22/3	9.5	74	4.54	168	2.88	3.69	0.780	40	
23/3	18.3								
24/3	15.6	76	4.25	156	2.99	3.67	0.815	55	
25/3	15.6	72	4.31	152	3.15	3.52	0.890	50	
26/3	17.8	73	4.78	158	3.12	3.31	0.940	50	
27/3	20.5	73	4.57	163	3.42	3.56	0.962	40	
28/3	24.4								
29/3	24.4	73	4.54	190	3.20	4.19	0.765	45	Noise
30/3	21.1								
31/3	21.1	74	4.35	167	2.89	3.85	0.750	55	
1/4	8.3	72	4.72	197	2.02	4.18	0.724	50	Noise
2/4	10.6	72	4.75	197	3.11	4.15	0.749	48	Tired
3/4	15.6	74	4.46	185	3.09	4.16	0.742	45	
4/4	16.7	71	4.50	176	3.00	3.91	0.764	45	
5/4	17.8	74	4.43	168	2.90	3.80	0.769	55	
7/4	20.5	74	4.66					40	
8/4	18.9	73	4.90	189	2.89	3.85	0.750	65	Noise
9/4	21.7	72	5.12	172	2.80	3.35	0.836	40	
10/4	19.4	70	4.32	166	3.84	3.85	0.737	45	
11/4	14.4	72	5.56	188	2.61	3.38	0.772	442	
12/4	18.3	72	4.92	197	2.94	4.00	0.735	40	
13/4	20.0								
14/4	21.1	73	4.93	178	2.99	3.62	0.826	40	
15/4	20.0	70	4.82	165	2.99	3.42	0.874	40	
16/4	20.0	78	5.16	175	3.15	3.38	0.933	40	
17/4	23.3	75	5.44	170	3.21	3.13	1.025	40	
18/4	17.8								M_1
19/4	18.3	72	4.90	198	3.11	4.04	0.771	50	
20/4	20.0								
21/4	21.1								
22/4	22.8	70	4.72	177	3.22	3.76	0.855	45	
23/4	24.4	71	4.70	179	3.52	3.82	0.922	45	
24/4	23.9								
7/5	22.9	70	4.44	177	3.01	4.00	0.753	50	
8/5	20.0	69	4.76	175	2.96	3.68	0.805	50	
9/5	22.2	70	4.15	155	2.88	3.74	0.769	40	Nearly asleep
10/5	18.9	69	4.10	150	2.50	3.60	0.695	40	
11/5	16.1								
12/5	20.0	76	5.10	191	2.84	3.75	0.756	50	
13/5	22.2	73	4.80	184	2.84	3.83	0.740	60	
14/5	23.9	72	4.30	176	2.88	4.08	0.707	45	
15/5	19.4	69	5.16	190	2.97	3.68	0.808	50	Fever p.m.
16/5	20.5	70	5.13	193	2.94	3.76	0.782	45	Fever p.m.

TABLE 6—*Concluded*

DATE	TEMPERATURE	PULSE	LITERS VENTILATION	CC. O_2	PER CENT CO_2	PER CENT O_2	R.Q.	MINUTES REST	NOTES
17/5	23.9	80	4.58	182	2.86	3.96	0.721	40	Fever p.m.
18/5	23.3	78	5.26	194	2.79	3.70	0.754	45	Fever p.m.
19/5	26.2	72	5.06	193	3.06	3.79	0.807	50	Fever p.m.
20/5	27.2	78	5.01	171	2.94	3.42	0.859	60	Fever p.m.
21/5	20.0	76	5.17	166	2.84	3.21	0.840	90	M_1
22/5	21.7	68	4.90	178	2.84	3.63	0.782	40	Excited
23/5	23.3								
24/5	26.2	70	4.82	184	2.90	3.83	0.757	40	
25/5	27.2								
26/5	27.2	69	5.04	193	2.92	3.84	0.734	60	
27/5	27.8	76	4.70	189	3.10	4.03	0.770	40	
28/5	27.8	66	3.60	180	3.61	4.99	0.724	55	
29/5									
30/5	28.9	66	3.82	183	3.74	4.81	0.780	50	

SUMMARY

1. This series includes 96 experiments on one subject and 80 on another made within a period of four months.

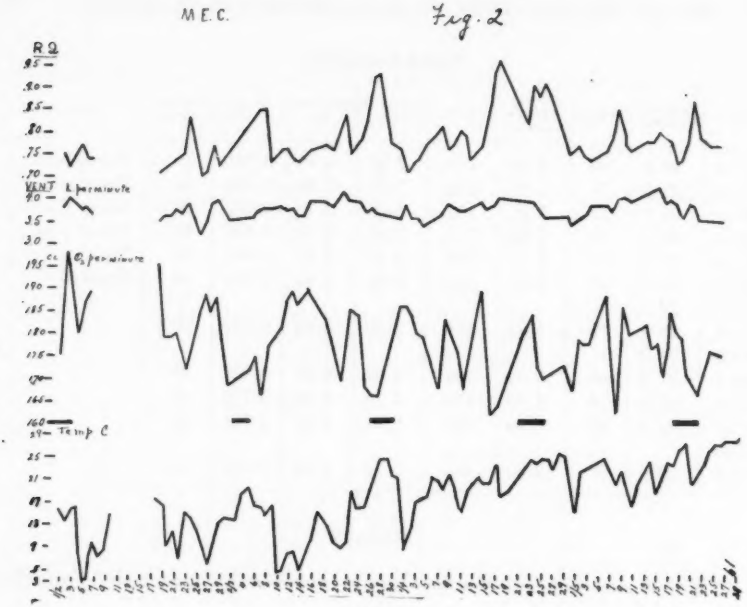
2. The average basal metabolism is 5 to 8 per cent below the Harris-Benedict standard.

3. Basal metabolism is about 5 per cent higher in cold weather (3 to 10° C.) than in hot (20 to 27°C.).

4. Average variation is ± 4.4 per cent for one subject, +5 per cent and −6.3 per cent for the other. The maximum variations are respectively +12.8 and −9.5 and +8.8 and −14.4 per cent.

5. Basal metabolism of both subjects is low on the first or second day of menstruation and often throughout the period. There is frequently a premenstrual rise and intermenstrual minimum. The difference between high and low levels amounts to 5 per cent.

6. Pulse rate is steady and shows no correlation with either temperature or menstruation.



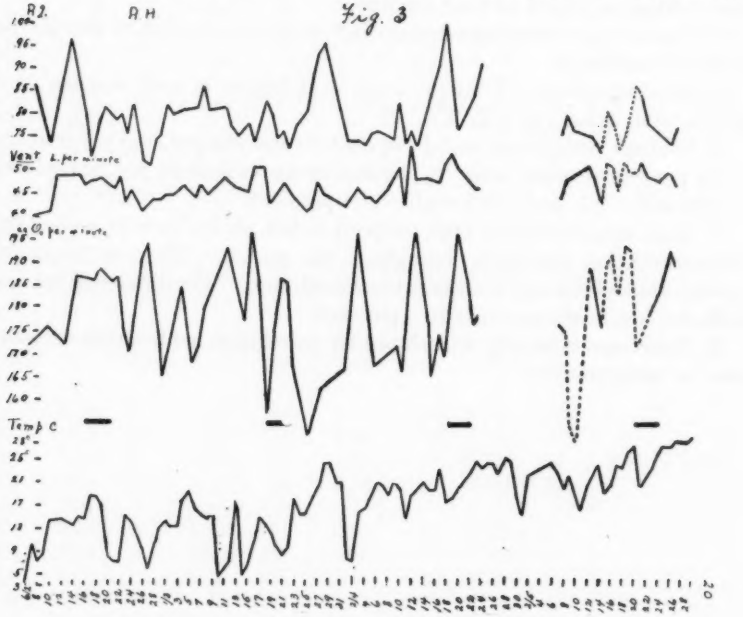


Fig. 2. Basal metabolism M. E. C. Dotted lines indicate questionable records (see table 5). Heavy blocks indicate menstrual periods.

Fig. 3. Basal metabolism R. H. Dotted lines indicate questionable records (see table 6). Heavy blocks indicate menstrual periods.

BIBLIOGRAPHY

(1) Blunt and Dye: Journ. Biol. Chem., 1921, xlvii, 263.
(2) Boothby and Sandiford: Physiol. Rev., 1924, iv, 69.
(3) Collett and Liljestrand: Skand. Arch. f. Physiol., 1924, xlvi, 17.
(4) Gephart and DuBois: Arch. Int. Med., 1916, xvii, 803.
(5) Kunde: Journ. Metab. Res., 1923, iii, 399.
(6) Liljestrand: Skand. Arch. f. Physiol., 1916, xxxiii, 153.
(7) McCann: Medicine, 1924, iii, 1.
(8) Moore and Cooper: This Journal, 1923, lxiv, 405.
(9) Rowe and Eakin: Cal. State Med. Journ., 1921, xix, 320.
(10) Snell, Ford and Rowntree: Journ. Amer. Med. Assoc., 1920, lxxv, 515.
(11) Wakeman: Journ. Biol. Chem., 1923, lvi, 555.
(12) Wiltshire: Lancet, 1921, ii, 388.
(13) Zuntz: Arch. f. Gynäkol, 1906, lxxviii, 106.

LEAD STUDIES

IV. Blood Changes in Lead Poisoning in Rabbits, with Especial Reference to the Stippled Cells

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Received for publication June 25, 1924

Stippling, or punctate basophilia, is a marked phenomenon of lead poisoning. By this term is meant the presence in certain red cells of discrete basophilic granules. Polychromatophilia is the term used to designate that quality of certain red blood cells of staining doubly with acid and basic dyes, which causes them to assume a diffuse red-violet color when smears, fixed in alcohol, are stained with eosin and methylene blue. The reticulum is a fine irregular granulo-filamentous network which appears in certain red cells when the blood is stained supravitally with any one of a fairly large number of basic dyes.

These three distinct basophilic phenomena were described as early as 1885 by Ehrlich (1). The voluminous literature which has appeared about them has recently been summarized by Pappenheim (2), Naegeli (3) and Ferrata (4), so it will not be dealt with here.

Polychromatophilia and reticulum occur normally in all young red blood cells. Stippling on the contrary is not a normal constituent of young red cells, but is found in certain embryological stages and in certain pathological anemias, one of the most important of which is that of lead poisoning.

In a previous paper (5) it was concluded that the polychromatophilia is due to the presence of a basophilic substance which is distributed uniformly through the hemoglobin-containing part of the erythrocyte and that the reticulum is formed by the union of this basophilic substance with a supravital stain. In the same paper it was suggested that stippling is the aggregation of this basophilic substance into small granules in the cell.

Sabrazès, Bouret and Léger (6) and many subsequent observers have shown that stippling occurs in the blood of rabbits and some other laboratory animals when they are poisoned with lead. It is well known that production of a secondary anemia in animals causes an increase in the number of young red blood cells in the circulating blood. If stippled

cells are young red cells in which the basophilic substance is aggregated into small intracellular masses, then the number of stippled cells appearing in the blood of animals after a given dose of lead should vary directly with the number of young red cells present in the circulating blood at the time the lead was given. In order to provide experimental evidence on this point the blood changes in normal and anemic rabbits were studied after experimental lead poisoning.

Experiments. In the first experiment four normal adult rabbits of approximately the same size were used. From two of these animals 25 cc. of blood were taken from an ear vein three times a week for two weeks. Then each rabbit received by stomach tube 0.7 gram lead acetate dissolved in water. The blood was examined for stippled cells daily during the three weeks after administration of the lead.

In the second experiment six normal adult rabbits were given freshly precipitated lead carbonate subcutaneously in doses of from 0.2 to 0.4 gram. These rabbits were observed for periods of from seven to eight months: the blood was examined weekly; injections were repeated at intervals of six to eight weeks. Each rabbit received a total of 1.6 gram of lead carbonate during the period of observation.

In the third experiment eighteen normal adult rabbits were used. These were studied in three groups of six each, the individuals of each group being as near the same size as possible. Three rabbits of each group were rendered moderately anemic by repeated bleeding, and three were kept as normal controls.

From each of the first group of rabbits 25 cc. of blood were taken eight times in three weeks; then on the same day 15 cc. of blood were taken and lead injected. In the second group 25 cc. of blood were removed seven times in two weeks, and lead was given on the third day after the final bleeding. Animals of the third group were each bled (25 cc. at a time) three times a week for two weeks; then twice a week for two weeks; and received lead on the tenth day after the final bleeding.

In the case of the normal rabbits daily blood counts were made at least three days before lead was given. After blood examinations had been made each rabbit received by stomach tube 1.0 gram of lead acetate dissolved in 20 cc. of water. The blood of each was examined the next day (about 24 hours later) and daily for periods varying from eleven days to three weeks.

Technique. In the first experiment cover-glass smears of blood were dried in air, fixed in methyl alcohol for 30 to 60 seconds, and stained in Manson's methylene blue (methylene blue medicinal 2.0 gram.; borax 4.0 gram.; water 100 cc.; diluted with 20 volumes of water). The smears were stained for half a minute, washed with water, dried in the air and mounted in cedar oil. Under the microscope the body of the red cells is

seen to vary in color from pale yellow to light blue, and the stippling is deep blue. The number of stippled cells present in fifty fields (oil-immersion lens) of a well-stained preparation of the blood from each rabbit was determined daily. So far as possible only those fields containing approximately the same number of cells were examined.

In the second and third experiments red and white cell counts were made in the usual manner. To avoid errors due to differences in apparatus the same pipettes and counting chamber were used throughout. Reticulated cell counts, made by the method of Robertson (7), were recorded as per cent of the total number of red cells. For counting stippled and polychromatophilic cells cover-glass smears were dried in air, fixed in methyl alcohol for thirty minutes, stained 20 to 30 minutes in Unna's alkaline methylene blue (diluted 1 part in 15 with water), washed in water, dried and mounted in cedar oil. By this method mature red cells are stained greenish yellow, the polychromatophilic cells red-violet, and intracellular stippling deep violet. The size of stippling varies from the limit of microscopic vision to that of coarse granules larger than those in eosinophilic leucocytes. A differential count of stippled and polychromatic cells can be made from these smears. All transitions between polychromatophilia and stippling may occur in a single smear, particularly after administration of lead, and exact differentiation is, therefore, open to some error. In this investigation only red cells containing three or more definite discrete blue granules were considered as stippled cells.

By making percentage counts of the polychromatophilic and stippled cells in stained smears, and a reticulated count of the same blood by Robertson's method, it was determined that the percentage of reticulated cells is approximately equal to the sum of the percentages of stippled and polychromatophilic cells. The reticulated counts were slightly higher than this sum because staining reactions in fixed smears are less delicate, and because, as Hawes (8) suggested, cells containing only a small amount of basophilic substance are not differentially stained. In table 1 are given differential counts from ten smears chosen at random, and reticulated counts of the same blood as determined by Robertson's method. These ten determinations of reticulated counts average 1 per cent higher than counts of stippled and polychromatophilic cells made from smear preparations. As this difference is well within the percentage error of the technique, the reticulated count probably represents more accurately the total number of young red cells in the blood.

First experiment. In the blood of all four rabbits some stippled cells appeared on the first day after the administration of lead, and the maximum number was present on the second day. After the fifth day the count gradually decreased until at the end of the second week few stippled cells could be found. The maximum numbers in fifty microscopic fields of smears from the two normal rabbits were 62 and 90 re-

spectively, while in the two anemic rabbits the maximum counts of stippled cells in fifty fields were 237 and 240.

Second experiment. In rabbits in which lead carbonate was subcutaneously injected in an attempt to cause chronic lead poisoning, stippled blood cells did not appear until ten or fourteen days after the first injection and were never numerous, although a few were present throughout the period of observation. The red counts in some instances were slightly lowered; in others they remained within the normal range. The reticulated counts varied from 1 to 5 per cent. The white counts were always within the normal limits.

Third experiment. In this series lead acetate was administered by mouth to the rabbits. In four cases a dose of 1 gram proved fatal and in every animal this amount caused some symptoms of acute poisoning and the prompt appearance of stippled cells in the blood. For some days after receiving lead, food and water were always refused and the animals lost weight. The eyes were sunken and the ears pale. A detailed description of changes in the blood is given below.

TABLE 1

Comparison of counts of young cells in different specimens of blood made from stained smears and by Robertson's method

SPECIMEN	STIPPLED CELLS	POLYCHROMATOPHILIC CELLS	STIPPLED PLUS POLYCHROMATOPHILIC CELLS	RETICULATED CELLS	DISCREPANCY
	per cent	*per cent*	*per cent*	*per cent*	*per cent*
1	0.6	3.0	3.6	3.8	0.2
2	1.0	4.3	5.3	6.0	0.7
3	0.6	4.3	4.9	5.0	0.1
4	1.4	1.9	3.3	5.0	1.7
5	0.9	1.1	2.0	3.3	1.3
6	16.0	2.0	18.0	19.0	1.0
7	0.6	4.6	5.2	5.8	0.6
8	1.9	0.7	2.6	4.3	1.7
9	9.3	0.0	9.3	10.6	1.3
10	9.0	0.5	9.5	10.6	1.1
Average....	4.1	2.2	6.4	7.3	1.0

a. Changes in the number of the red blood cells. In the nine normal rabbits (fig. 1), administration of lead was followed in the first twenty-four hours by the sharp reduction of about 1,000,000 red blood cells per cubic millimeter of blood. This decrease continued progressively for three to five days and was usually followed by slow and slightly irregular recovery lasting two to four weeks. As a rule the initial fall was large; in some cases it amounted to over 2,000,000 red cells per cubic millimeter in the first 24 hours following the administration of lead (fig. 2). Variations among individual rabbits, however, were marked. Figures 2 and 3 show the extremes of variation among these animals. Three normal rabbits died before the ninth day.

Figures 4, 5 and 6 show the daily changes in the number of red and stippled cells per cubic millimeter of blood in the nine anemic rabbits. Each chart is the average for a group of three animals. In the first group (fig. 4) the fall in the red count is about the same as in the normal group but recovery is more rapid.

In the second group (fig. 5) with a short period between the last bleeding and the administration of lead, this fall is less than half of that in the normal controls. One rabbit of this group died on the third day.

Results obtained with the third group of anemic rabbits are shown in figure 6. With the anemia produced more gradually and a longer interval between the last bleeding and the administration of lead there followed a

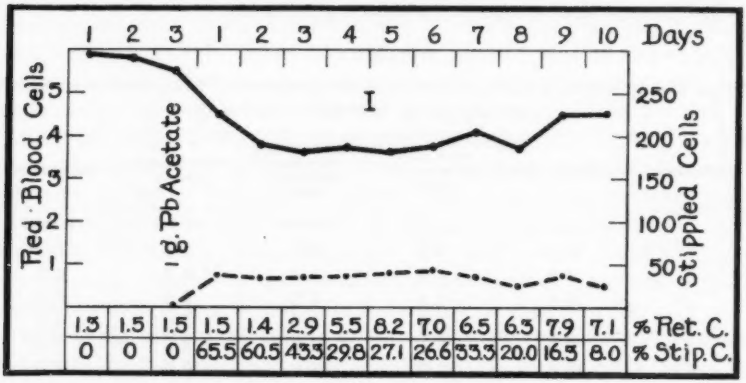

Fig. 1. The average daily red and stippled cell counts for a group of nine normal rabbits before and following a single large dose of lead.

In figures 1 to 7 inclusive, the solid line indicates the number of red blood cells, and the broken line the number of stippled cells per cubic millimeter. Ordinates for red blood cells 1,000,000, for stippled cells 50,000. Abscissae in days. The first line of figures at the bottom gives the daily percentage of reticulated cells. The second line of figures gives the percentage of the reticulated cells which were stippled.

sharp initial rise in the red blood count which was maintained at the higher level for two days.

Figure 7 shows the average changes in the number of red and stippled cells per cubic millimeter of blood in the nine anemic rabbits of this series after the administration of 1 gram of lead acetate. These changes are less than those exhibited by the nine normal rabbits (fig. 1) subjected to a similar dose of lead.

b. The reticulated cells. The percentages of reticulated cells present in the blood are shown at the bottom of figures 1 to 7. The average reticulated count for the nine normal rabbits during the preliminary period of observation was 1.5 per cent (fig. 1) and the extremes were 0.5 per cent

and 3.0 per cent. While the most marked destruction of red cells occurred on the first and second days, the increase in the percentage of reticulated cells did not begin until the third day after giving lead. In seven of these normal rabbits there was a slight fall in the percentage of reticulated cells on the first and second day. After this initial decrease there was an increase which as a rule was only moderate in degree. However, in two

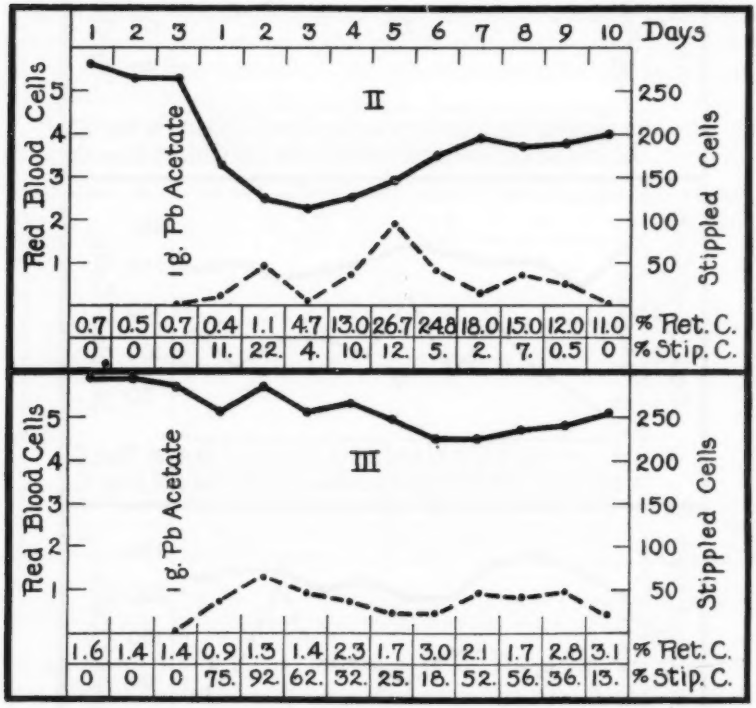

Fig. 2. A normal rabbit showing marked reduction in red cell count following a single dose of lead.

Fig. 3. A normal rabbit showing but slight reduction in red cell count following a single dose of lead.

cases the reticulated count rose rapidly: in one rabbit of the first group it was 0.7 per cent when the lead was given, then fell to 0.4 per cent on the first day, rose to 1.1 per cent on the second day, and three days later was 26.7 per cent. The average reticulated count for six of the normal rabbits on the fifth day was only 2.2 per cent. With these relatively low reticulated counts the appearance of nucleated red cells in the circulation,

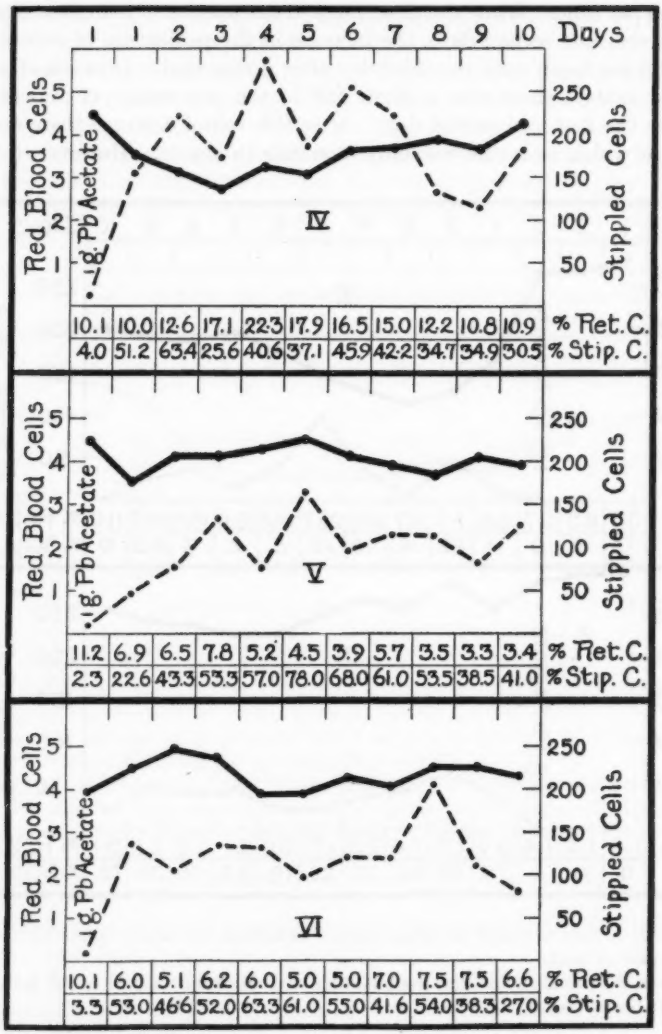

Fig. 4. The average daily red and stippled cell counts of a group of three rabbits rendered anemic by bleeding and then receiving a single large dose of lead.

Fig. 5. The average daily red and stippled cell counts of a second group of three rabbits rendered anemic by a somewhat more rapid course of bleeding and receiving a single large dose of lead three days after the last hemorrhage. One animal died on the third day following the administration of lead.

Fig. 6. The average daily red and stippled cell counts of a third group of three rabbits rendered anemic by repeated bleeding during a somewhat prolonged period and receiving a single large dose of lead ten days after the last hemorrhage.

suggested that the bone marrow was actively producing young cells but that these were being destroyed almost as fast as they were formed.

In the anemic rabbits (figs. 4, 5, 6 and 7) the changes in the reticulated counts were especially interesting. The average for the nine rabbits (fig. 7) shows that the number of reticulated cells never rose much above that present at the beginning of the experiment because of the ratio between the destruction of blood and the production of red cells by the bone marrow. That the marrow was active is demonstrated by the appearance of numbers of nucleated red cells in the circulation which, in some cases, amounted to over 10,000 per cubic millimeter, on the third and fourth days after receiving lead.

The three groups of anemic rabbits present marked differences in the changes in the number of red cells per cubic millimeter of blood after

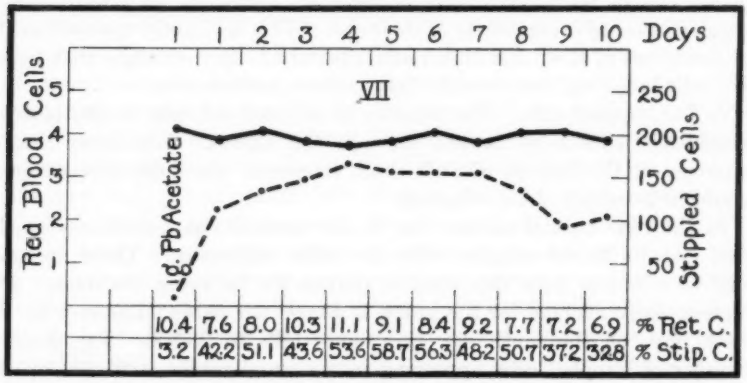

Fig. 7. The average daily red and stippled cell counts of a group of nine rabbits rendered anemic by bleeding and then receiving a single large dose of lead.

the administration of 1 gram of lead acetate. In group I (fig. 4) the lead was given at the height of bone narrow activity. Here the red count fell rapidly and did not attain its original level until the eleventh day, while the reticulated count rose rapidly to 22.3 per cent on the fourth day and continued high for two weeks. In group II (fig. 5) the red count fell on the first day and then by a gradual rise returned to its original level by fifth day. The reticulated count, which was 11.2 per cent just before lead was given, fell sharply to 6.9 per cent on the first day and continued to fall irregularly during the two weeks. In the third group (fig. 6), with ten days' respite after the last bleeding, the red counts rose about 1,000,000 during the first two days while the reticulated counts fell from 10.1 per cent to 5.1 per cent during the same period. This paradoxical behavior suggests that the reticulated cells are destroyed at least twice as fast as the normal cells.

In several animals the red cells showed marked fragmentation and the anisocytosis and poikilocytosis was comparable to that of severe pernicious anemia. In supravital preparations, in which the cells were in isotonic medium, the process of cellular destruction could be observed. The cells were not laked but, while retaining their hemoglobin, seemed to soften, lose their regular contour, and assume bizarre shapes with irregular rounded processes separated from the parent cell by constriction. Sometimes the cells simply elongated and broke in two. The fragments rounded out or again divided, but in no case did there seem to be any tendency for them to lose their hemoglobin. This process is similar to that described by Rous and Robertson (9) as the normal method of destruction of blood. In my experience, these phenomena have been limited to mature erythrocytes and have not involved either polychromatic or stippled cells. Young microcytes of both types were occasionally seen, but they were always regular in contour regardless of their size. This is directly opposed to the observations of Rous and Robertson from which they conclude that young red cells break up more readily than mature erythrocytes.

c. The stippled cells. The number of stippled red cells in the blood is graphically shown by broken lines in the figures. The lower row of numbers at the bottom of each chart represents the daily percentage of young cells which show stippling.

In the nine normal rabbits (fig. 1) the average count increases on the first day to 39,500 stippled cells per cubic millimeter. There are only slight variations from this number during the following ten days. The highest daily average for the group is 43,000 per cubic millimeter on the sixth day; on the tenth day this figure falls to 23,300. The greatest number of stippled cells appearing in a normal rabbit is 95,100 on the fifth day: at this time the percentage of reticulated cells is 26.7; and 12 per cent of the young cells are stippled. The rabbit with the smallest number of stippled cells died on the ninth day: the highest stippled cell count in this animal is 11,760 per cubic millimeter on the third day.

The numbers of stippled cells per cubic millimeter in the nine anemic rabbits are shown in figure 7. It is to be noted that a small number of stippled cells was present in the blood of five of these animals before any lead was given. The stippled counts in these five cases range from 11,000 to 32,000 per cubic millimeter, while the average for all nine rabbits is 12,000. A sharp rise to 113,000 on the first day after administration of lead is followed by a more gradual rise to the maximum average of 166,000 on the fourth day. This high level was maintained for three days before the beginning of a gradual decline which reduced the count to 109,000 on the tenth day. On the whole, the average stippled counts for the nine anemic rabbits of this series are slightly more than three times as great as those for the normal group (figs. 1 and 7). The percentages at the bottom

of the figures demonstrate that during the first two days smaller percentages of the young cells in anemic rabbits show stippling, but that after the third day the relationship is reversed, i.e., there is less stippling in the control group.

Examination of the experimental results shows that many more stippled cells are found in the blood of all the anemic rabbits than in the blood of the normal "leaded" animals, the greatest number appearing in the group which received lead directly after the last bleeding (fig. 4). A comparison of the percentage figures at the bottom of each figure shows that the differences in the number of stippled cells in the three groups is due almost entirely to changes in the number of reticulated cells rather than to differences in the per cent of the young cells which show stippling. The highest count of stippled cells in any rabbit was 399,000 per cubic millimeter: this was obtained on the fifth day in one animal of group I (fig. 4). Of eight anemic rabbits the animals with the minimal reaction had a stippled cell count of 195,000 per cubic millimeter on the sixth day. This is slightly more than twice the highest number found in any normal rabbit. The ninth anemic rabbit (group II) died on the third day with only 34,000 stippled cells per cubic millimeter. All the reactions of this rabbit were, however, atypical and it is probable that little lead was absorbed.

The per cent of the young cells showing stippling bears no constant relation to the apparent degree of destruction of blood. Figures 2 and 3 record graphically the extremes of this destruction in the normal series. The most marked reduction of the red count occurs in an animal with stippling in a maximum of 22 per cent of the young cells; in the rabbit with the least destruction of blood, 92 per cent of the young cells were stippled on the second day. However, a rabbit of the first normal group, which died on the third day, had the red count reduced to 3,256,000 on the second day, while 93 per cent of the young cells were stippled. Therefore, in the control animals there is evidence that the presence of stippling in a large percentage of young cells may be accompanied by either marked or very slight reduction in the red blood count. The same holds true for the anemic rabbits. More detailed comparison of the findings with anemic and the normal rabbits, however, shows that as a rule when the reticulated count is high a smaller per cent of young red cells becomes stippled than when only a few reticulated cells are present. About the same proportion of nucleated and reticulated red cells appear to be stippled.

d. The white blood cells. In figure 8 are given the average daily white cell counts for the nine anemic and the nine normal rabbits. The administration of lead was followed by a sharp rise in the white count in both groups. In the normal rabbits the maximum was 58,500; in the anemic, 42,100 per cubic millimeter.

Comparison of the normal and anemic groups (fig. 8) shows that leucocytosis in the anemic rabbits reaches its maximum sooner than in the normal rabbits, but that it does not reach so high a level and is not sustained for so long a time. The figures in the chart include those for nucleated red cells present on the various days. A shower of nucleated cells usually appears on the third or fourth day and then quickly disappears, but a few nucleated cells remain present throughout the ten day period. They are more numerous in the anemic than in the normal rabbits.

Daily differential counts were not made for all the rabbits and exact figures are therefore not available. However, from the number of counts made from both normal and anemic rabbits it was determined that the administration of lead is usually followed by slight reduction in the percentage of polymorphonuclear elements and rather marked reduction

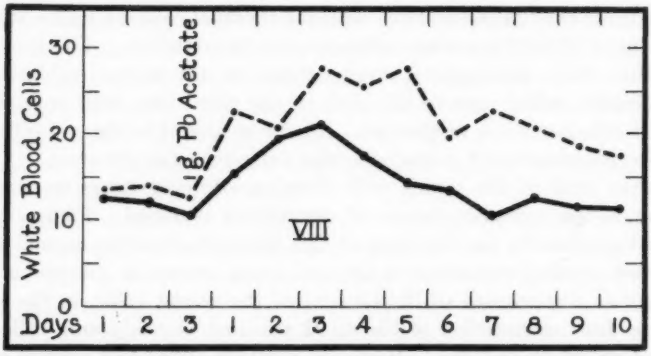

Fig. 8. The average daily leucocyte count for the nine anemic and the nine normal rabbits: solid line—anemic group; broken line—normal group; ordinates in thousands; abscissae in days.

in the number of small lymphocytes. The large mononuclear cells and large lymphocytes were found to be increased in number and on the third or fourth day their sum is usually 25 to 30 per cent of the white cells. Large numbers of smudges (degenerating leucocytes) were seen in the smears from the poisoned animals, and the presence of these as well as the decrease in the small lymphocytes, suggests that lead acts as a destructive agent on both types of white cells. No constant differences appear between the differential counts of normal and anemic groups except that in the anemic animals there is a moderate increase in the percentage of large mononuclears and lymphocytes before lead is given.

SUMMARY AND DISCUSSION. The experiments in which relatively insoluble lead carbonate was injected subcutaneously show that it is possible for rabbits to absorb lead for long periods of time without showing more

than very slight changes in the blood. There is no evidence of any cumulative action and the only constant abnormality in the blood is the presence of a few stippled cells.

Those rabbits which were given the freely soluble lead acetate by mouth at once developed acute poisoning with marked blood changes. In the normal rabbits there was a definite reduction in the number of red blood cells. The appearance of increasing numbers of young red cells in the blood after the administration of lead indicates that anemia is due to destruction of blood rather than to suppression of the activity of bone marrow. Laked erythrocytes (shadows) were not seen in usual numbers, but many irregularly shaped red cells and microcytes were often seen, and at times division of the red cells was observed *in vitro*. Fragmentation consisted in a simple breaking of the cell into two or more hemoglobin-containing masses of irregular size and shape; and there was no tendency for it to proceed to such a point that the particles were extremely fine (blood dust of Robertson). These observations lead to the belief that the destruction of blood in lead poisoning is due to fragmentation rather than to hemolysis. Post-mortem studies contribute additional evidence to support this opinion in that the endothelial cells of the liver and spleen of the poisoned animals were loaded with hemoglobin-containing fragments.

The same destruction of blood undoubtedly occurs in the anemic rabbits but the reduction in the red count is less marked because the hyperactive bone marrow produces an abnormally large number of red cells and thus masks the actual loss of erythrocytes. In five of the anemic rabbits there was not only compensation for destruction of blood but the administration of lead was actually followed by a rise in the red count which lasted from one to five days.

There was a transient decrease in the reticulated count in five of the normal rabbits and a permanent decrease in the reticulated counts in five of the anemic rabbits after the administration of lead. The abrupt fall in the reticulated count in the second and third groups of anemic rabbits is evidence that lead exercises a more destructive effect on young than on mature erythrocytes, in spite of the fact already noted that fragmentation has not been observed in any reticulated red cells. The later rise in the reticulated counts which occurred in all of the normal and in four of the anemic rabbits is of course caused by increased activity of the bone marrow and the diminishing toxemia from the single dose of lead.

In the third group of anemic rabbits, the red count increased only slightly during the ten days between the end of bleeding and "leading;" but a sharp increase in the red counts coincident with an abrupt fall in the percentage of young cells in the blood followed the administration of lead. This suggests that during the rest period the stimulated hematopoietic tissues form a considerable reserve of mature red cells which are stored,

in spite of the moderate secondary anemia; and that in response to the strong stimulus brought to bear by acute lead poisoning these stored cells are thrown into the circulation.

Stippled cells always appeared within twenty-four hours after "leading" and could be observed for two weeks or more. They were about three times as numerous in the anemic as in the normal rabbits, and their number after "leading" was in great measure dependent upon the number of reticulated cells present in the blood when lead was given. This is further evidence that the stippled cell is an altered young red blood cell. The percentage of the young blood cells which showed stippling,—that is, the ratio of stippled to non-stippled polychromatophilic red cells—was as a rule higher when the blood contained smaller numbers of young red cells. There is apparently no relation between either the total number of stippled cells or the per cent of young cells which became stippled and the diminution of the red cell count. Rabbits and guinea pigs easily developed this stippling in experimental lead poisoning, but in a series of cats severely poisoned with lead no stippling could be demonstrated. A group of hens studied by Dr. A. S. Minot in this laboratory also showed no stippling of the erythrocytes on repeated examination, in spite of the fact that practically every red cell in the chicken contains some basophilic substance. This has also been observed by Meyer and Speroni (10). It is to be noted that examination of the bone marrow of a number of rabbits, in whose blood large numbers of stippled cells were present, revealed no stippling of the cells within the marrow. Probably, then, stippling is a change in the basophilic substance of the young red cell which takes place after the cells enter the circulation. It is characteristic of pathological anemia and is undoubtedly degenerative in nature. However, stippling is seen in embryonic blood and five of the anemic rabbits of this series showed it in moderate degree before receiving any lead.

CONCLUSIONS

1. Experimental lead poisoning in rabbits is consistently followed by the appearance of stippled cells in the blood. These are not found in chickens or cats.
2. Far more stippled cells appear in the blood of anemic than of normal rabbits after ingestion of lead.
3. The stippled cell is a young red cell.
4. The anemia of lead poisoning is due to actual rapid destruction of red cells in the blood stream and not to inhibition of the activity of bone marrow.
5. Young red cells are destroyed more rapidly than mature cells.
6. Leucocytosis occurs during experimental acute lead poisoning.

BIBLIOGRAPHY

(1a) EHRLICH: Berl. Klin. Wochenschr., 1880, xvii, 405.
(1b) EHRLICH: Berl. Klin. Wochenschr., 1881, iii, 43.
(1c) EHRLICH: Charite-Annalen, 1885, x, 136.
(2) PAPPENHEIM: Folia Haematol., 1919, xxiv, 86.
(3) NAEGELI: Blutkrankheiten und Blutdiagnostik. Berlin u. Leipzig., 1919, 140.
(4) FERRATA: Le Emopatie. Milan, 1919.
(5) KEY: Arch. Int. Med., 1921, xxviii, 511.
(6) SABRAZES, BOURET ET LEGER: Journ. de Phys. et Path. Gen., 1900, ii, 497.
(7) ROBERTSON: Journ. Exper. Med., 1917, xxvi, 221.
(8) HAWES: Boston Med. Surg. Journ., 1909, clxi, 493.
(9) ROUSE AND ROBERTSON: Journ. Exper. Med., 1917, xxv, 651, 665.
(10) MEYER UND SPERONI: Münch. Med. Wochenschr., 1906, liii, 796.

A STUDY OF TESTICULAR VOLUME CHANGES

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Received for publication June 18, 1924

Comparatively little attention has been given to vasomotor changes, as well as to other possible motor activities of the testicle. The mass of literature bearing on the functional activities of this group of organs deals either with the development of the reproductive cells or with testicular hormone.

On anatomical grounds one would expect to be able to observe vasomotor changes and possibly other movements in the testicle following the introduction of certain drugs, and also after stimulation of the nerves supplying these tissues. This last phrase is more applicable if we include the epididymis as part of the testicle. More will be made of this point in connection with the detailed descriptions and statement of our own experiments.

The general course of the nerves supplying the testes of the rabbit and cat was worked out by Langley and Anderson (1). More recently Kuntz (2) has investigated this question with reference to the dog. The results of these observers indicate that the testicular nerve supply is derived from the lumbar cord, following the spermatic arteries to their terminations. Kuntz states that the hypogastric nerves supply fibers to the ductus deferens, but that they probably do not reach the testicle. We interpret this to mean that they do not go beyond the epididymis.

The exact terminations and the functional significance of these nerves is not so well worked out. Practically all agree that the blood vessels of the testicles are supplied by the nerves, and consider them vasomotor in function. The question as to whether any nerves penetrate the membrana propria is apparently an open one. Letzreich (3) was perhaps the earliest to investigate this question. He reported the finding of nerve terminations in the deeper layers of the seminal epithelium. Cavalie (4) and also Loisel (5) agree with this finding. Timofeew (6) could find no such endings, and more recently Kuntz reported negative results. There seems to be no doubt that the ducts leading away from the testicles are supplied with nerves which are motor in function. The results obtained by Macht (7) and by Waddell (8) bear out this conclusion. The fact that the epididymis is supplied by motor nerves is of particular importance because of its close relation to the testicle proper, and unless particular care is taken

to eliminate it one is likely to be misled in intepreting volume changes of the testicle.

The work reported in the following pages was undertaken for the purpose of making further inquiry into the vasomotor activities of the testicle, and also to determine if this organ manifests any other type of motor changes.

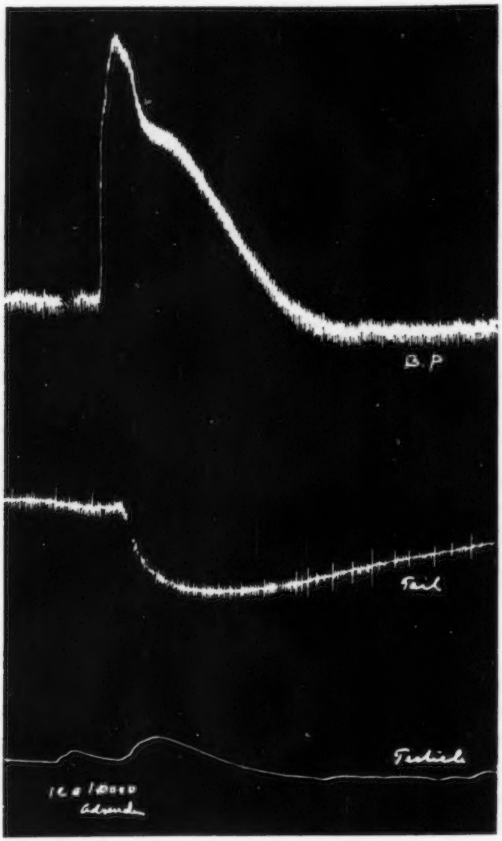

Fig. 1. Showing the effect of 1 cc. of 1:10,000 epinephrin on blood pressure, tail volume, and testicular volume.

Dogs were used for the experiments throughout. Testicular volume changes, made with an oncometer and tambour system, were recorded along with carotid blood pressure changes, and in some instances with volume changes of the tail or hind leg. Direct observations on the blood flow through the periphery of the testicle were made with the aid of a bi-

nocular microscope. In one series of experiments the testicle was perfused with warm oxygenated saline and dextrose at a constant pressure and the volume changes of the organ recorded. In the experiments with the living animal, with the organs in situ, either barbital or ether was used as an anesthetic. We were not able to note any significant differences in the response with these anesthetics except that in the case of barbital the reflex excitability of the animal was better.

An attempt was first made to determine the relation between systemic blood pressure and testicular volume. The pressure changes were brought about by the introduction into the vascular system of epinephrin, nitrites and pilocarpin.

The injection of 1 cc. of 1:10,000 epinephrin was invariably followed by a primary rise in testicular volume. Figure 1 is a reproduction of a typical record. It will be noted that the testicular volume increased with the sharp rise in blood pressure, fluctuated, then decreased gradually to a level below the normal, then gradually recovered. The tail volume decreased sharply with the rise in blood pressure, then gradually recovered its normal level. Figure 2 is another record without the tail volume showing the initial increase in volume better and also the subsequent diminution.

The introduction of 1 mgm. of nitroglycerin was followed by a very slight primary fall in testicular volume coördinate with the sharp fall in blood pressure. This was followed by a more marked and lasting increase in volume. Figure 3 is a reproduced record of the results of this procedure. It will be noted that the tail volume shows no initial decrease nor does the increase in volume hold as in case of the testicle.

Figure 4 is a record of the results following the injection of 3 mgm. of pilocarpin hydrochloride into an animal weighing 12 kgm. It will be noted that there was a sharp decrease in testicular volume coördinate with the initial fall in blood pressure, followed by a definite and sustained increase in volume. The variations in tail volume were of the same general character but not so marked.

The results recorded above would indicate that testicular volume and presumably the blood flow through that organ are largely dependent upon systemic blood pressure. It also seems clear that the blood vessels of the testicle are supplied with vasoconstrictor nerves and that they manifest definite variations in tone.

In several animals the blood flow through the peripheral vessels of the testicle was observed through a binocular microscope. The results of these observations checked very well with the volume records. After the injection of 1 cc. of 1:10,000 epinephrin the vessels first became definitely larger; in fact, some which could not be seen before came plainly into view. Following this initial engorgement there was a definite shrinkage corresponding closely with a diminution in volume. Local application of epi-

nephrin brought about a marked shrinkage in the blood vessels in the field under observation which was not preceded by any dilatation or engorgement. This shows that the initial effect is not local but dependent upon an outside factor.

In order to further test out this hypothesis a series of perfusion experiments was run. The testicle along with a considerable length of the sper-

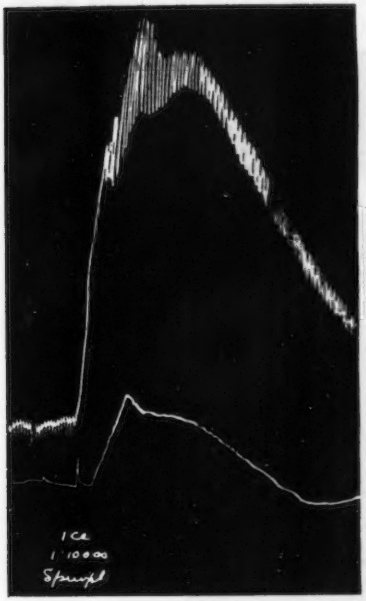

Fig. 2

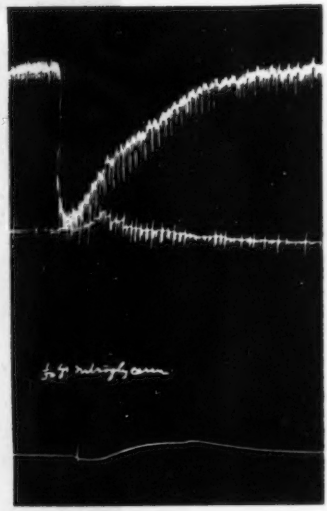

Fig. 3

Fig. 2. Blood pressure and testicular volume after epinephrin. Upper line, blood pressure, lower, testicular volume. The abrupt changes in the first part of the lower tracing are due to a jarring of the oncometer accidentally while preparing for and making the injection.

Fig. 3. Showing the effect of an injection of $\frac{1}{500}$ grain of nitroglycerin on blood pressure, tail volume and testicular volume. Upper tracing, carotid blood pressure, middle, tail volume, and lower, testicular volume.

matic artery and vein was removed from the animal. A cannula was inserted into the spermatic artery. The cannula was then connected with a tube leading from a pressure bottle containing saline and dextrose which was oxygenated by means of a constant stream of air. The apparatus was so arranged that the fluid from the bottle was made to run through a system of glass and rubber coils immersed in a water bath and kept at such a temperature so that the fluid was delivered from the nozzle of the

cannula at a temperature of 38°C. The testicle so prepared and connected was enclosed in an oncometer and the whole outfit submerged in a saline and dextrose bath which was kept at a temperature of 38°C. The artery and vein were long enough so that the outflow from the testicle fell outside of the bath vessel. The drugs were introduced into the system just above the warming coil. By this system we hoped to avoid changes due to variations in pressure and temperature, the results depending entirely upon changes in the tone and caliber of the vessels in the encased organ.

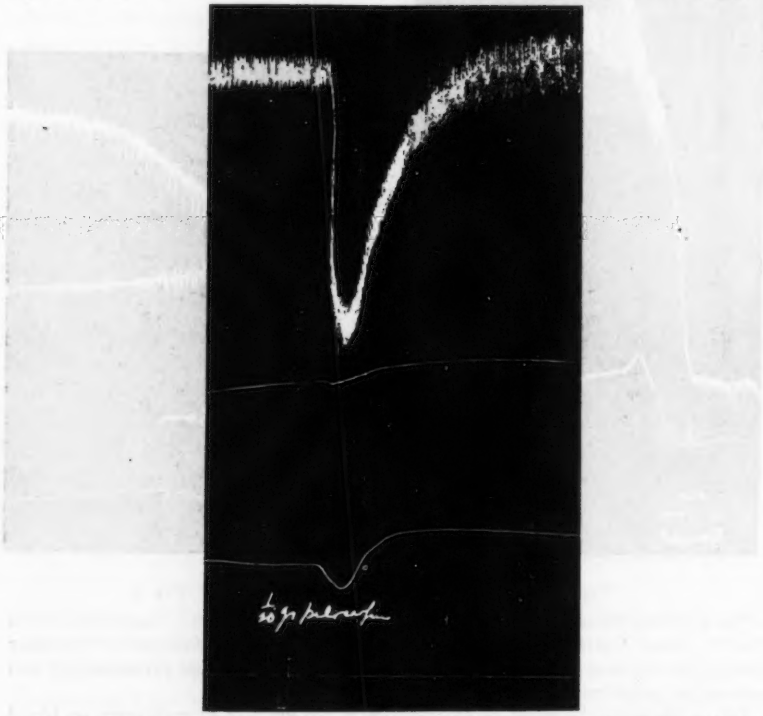

Fig. 4. Showing the effect of 3 mgm. of pilocarpine hydrochloride on carotid blood pressure (upper tracing), tail volume (middle), and testicular volume (lower).

Figure 5 is a record of the results of the perfusion of the testicle with epinephrin. There was a sharp decrease in testicular volume. One notices at the very beginning a slight increase, but it was found that such an increase could be entirely prevented if the drug was introduced slowly and at a pressure as nearly that of the fluid column as possible. We would interpret this slight initial change as being due entirely to mechanical factors and not to any change in the vessels. The evidence of a definite

constriction somewhere within the testicle was also further enhanced by the fact that following the drug there was a definite decrease in the rate of outflow of fluid from the spermatic vein.

When sodium nitrite was introduced into the perfusion fluid the testicle promptly increased in volume. Parallel with this the outflow from the vein also increased.

We were not able to obtain any definite changes in testicular volume following the introduction of pilocarpin into the perfusion fluid.

In making the studies and observations recorded above, one other phenomenon in particular attracted our attention. In a number of instances the testicle under observation manifested somewhat rhythmical

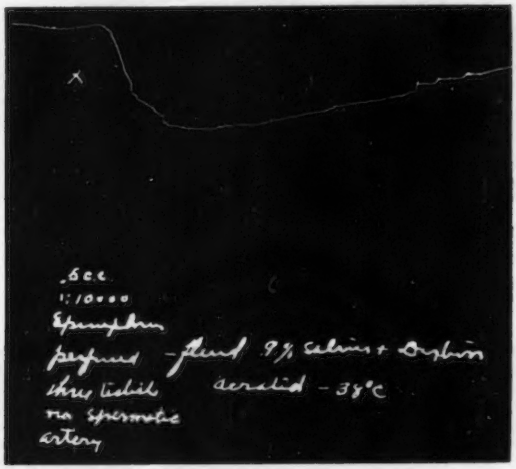

Fig. 5. Volume change in the testicle during its perfusion with epinephrin. At X the drug was introduced into the perfusion system.

variations in volume. This occurred in some instances before the administration of any drug, at other times after. A very pronounced result of this type was obtained in one of our animals after 3 mgm. of pilocarpin had been given. Figure 6 is a reproduction of the record. In this case it does not seem possible to ascribe these changes to variations in blood pressure. The tail volume shows no such variations, neither are the fluctuations in the blood pressure tracing of sufficient magnitude. Figure 7 is a record of such movements in an animal before the administration of any drug. Since we never observed such changes in any preparation in which the epididymis had been removed, we are inclined to ascribe these movements to it.

A survey of the literature indicates that observations on movements of the seminal ducts in dogs are not in strict accord. Waddell recorded

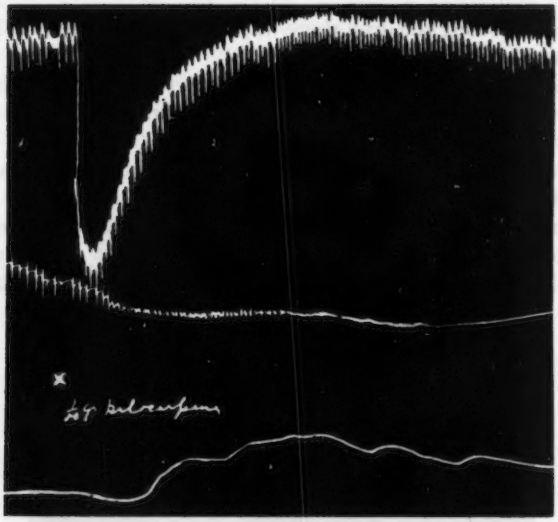

Fig. 6. Showing rhythmical volume changes in the testicle after 3 mgm. of pilocarpine hydrochloride. Upper tracing blood pressure, middle, tail volume, lower, testicular volume.

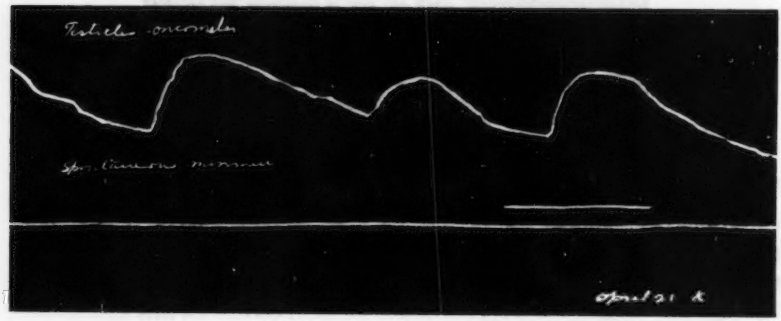

Fig. 7. Rhythmical volume changes in the testicle. No drug or artificial stimulation of any kind used.

rhythmical movements, but Macht was unable to obtain them. We made no attempt to record movements of isolated segments of any part of the seminal ducts, but in several of the perfusion experiments the vas deferens executed definite rhythmical movements. We have no criterion for comparing the rate of the movements recorded by Waddell with those

observed by us, or of the volume changes recorded. In our experience the waves came at the rate of about two each minute.

We next turned our attention to the study of testicular volume changes following electrical stimulation of the nerves going to the testicles and the seminal ducts. Records were obtained of the effects following stimulation of the hypogastric nerves and of the nerves running along the spermatic arteries.

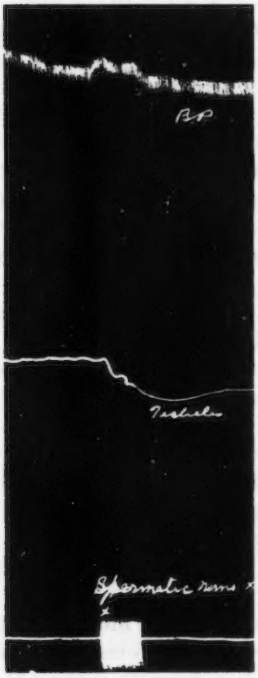

Fig. 8

Fig. 9

Fig. 8. Showing the effect of stimulation of spermatic nerves.

Fig. 9. Showing the effect of stimulation of the hypogastric nerves on blood pressure, (upper), and testicular volume, (lower).

Figure 8 is a record of the result of stimulation of a group of nerve fibers coming from the aortic plexus in the region of the origin of the right spermatic artery. These nerves were traced and found to accompany the artery. The testicular volume decreased sharply when they were stimulated. The central connections of these nerves were not severed. The slight rise in blood pressure was probably reflex. There was no initial increase in testicular volume as in the case of epinephrin injection and as

will be shown later following stimulation of the hypogastrics. We never recorded any rhythmical changes in volume of the testicle directly referable to spermatic nerve stimulation. The results are interpreted as being purely vasoconstrictor.

The results of hypogastric stimulation are more difficult of interpretation. The general character of the result is shown in figure 9. The initial increase in volume is shown in all our records. The succeeding fall is not so marked in many records and in some it did not occur at all. In the record here reproduced there is a remarkable parallelism between organ volume and carotid blood pressure. It is impractical to print records showing all types of responses obtained. Several generalizations may be noted here. The primary increases occurred regardless of the direction of the blood pressure. The secondary fall is somewhat commensurate with the fall in blood pressure and there is no decrease in volume at all in case there is no fall in blood pressure. The primary increase in volume occurs when the spermatic arteries are ligated and also when the central connections of the hypogastrics are severed, but does not occur when the epididymis is not within the oncometer.

Are the results outlined above to be interpreted as vasodilator effects, or are they due to other movements of the epididymis?

Attempts to answer the above question have been somewhat fruitless. It is difficult to conceive of any form of contraction of the epididymis which would record itself as a volume increase. The oncometer used was patterned somewhat after the Roy kidney oncometer. The outer part was made of a two ounce ointment box. Within this was placed another half box over which a thin membrane was loosely stretched. The air chamber was connected with the tambour through a $\frac{3}{16}$ inch brass tube which was soldered into the base of the inner shell. This tube passed through the outer shell and was held in place by a nut.

It occurred to us that during the reaction, the cremaster muscle might contract and pull the testicle outward, thus exerting some pressure on the rubber. This was shown to be a false assumption because cutting the cremasters made no difference in the results.

The final test experiment was carried out as follows: the testicles, including the epididymi, were enclosed in the oncometer and the hypogastrics isolated and stimulated. The usual initial increase in volume was recorded. The vessels along the seminal ducts were then ligated, care being taken not to include the nerves. On stimulation of the hypogastrics, the testicular volume increased in normal fashion. The spermatic arteries were then clamped and the hypogastrics again stimulated. The response of the testicles was very slight. The clamps were then removed from the spermatic arteries and the hypogastrics again stimulated. The response was of the normal type and magnitude. The central connections of the

hypogastrics were then severed and the peripheral ends stimulated. The result was practically identical with that following stimulation of the nerve with the spermatic arteries clamped off.

We would conclude that two factors enter into the results of hypogastric stimulation. One is a change in the epididymis due to efferent impulses and the other is due to changes in the systematic blood pressure, reflex in nature. Two hypotheses suggest themselves in explanation of the peripheral effect, neither of which can be definitely proved or disproved with the data at hand. One hypothesis is that the hypogastrics contain vasodilator fibers. In face of the fact that a slight initial increase in volume occurs with the blood supply shut off completely from the testicle and epididymis, it is conceivable that the vessels are normally in a state of tone and by their relaxation with the blood trapped led to a slight increase in volume. The other hypothesis is that the peripheral effect is due to an activity of the musculature of the seminal ducts. We are inclined to this view. Just what the mechanism is, we are not in a position to say.

The secondary effects of hypogastric stimulation seem to be clearly vascular inasmuch as they do not occur when the blood supply is shut off, or when the central connections of the hypogastric are severed.

SUMMARY AND CONCLUSIONS

The data presented bear mainly upon two phases of testicular and epididymis activity. On the one hand, some light is thrown upon the regulation of the blood supply and on the other, on the motor activities of the epididymis.

The evidence points clearly to the fact that there are vasoconstrictor fibers in the spermatic nerves. Under no conditions have we been able to obtain a sustained diminution in testicular volume with a high systemic blood pressure. This indicates that the blood supply to the testicle varies largely in proportion to the systemic blood pressure and that the vasoconstrictor mechanism does not play such an important rôle in the testicle as in some other organs, the kidney, for example.

No clear-cut evidence has been obtained for the existence of vasodilators in either the spermatic nerves or in the hypogastrics.

A rhythmical volume change in the epididymis has been recorded. This change appears independent of variations in blood pressure and has been interpreted to be due to muscular activity in the seminal ducts.

BIBLIOGRAPHY

(1) Langley and Anderson: Journ. Physiol., 1896, xix, 372.
(2) Kuntz: Anat. Rec., 1919, xvii, 203.
(3) Letzreich: Quoted by Kuntz.
(4) Cavalie: Compt. rend. Soc. de Biol., 1902, T. ii, s. iv, 289.

(5) Loisel: Ibid., 1902, T. liv, 346.
(6) Timofeew: Anat. Anz., 1894, Bd. ix, 342.
(7) Macht: Journ. Pharm. Exper. Therap., 1916, lx, 121.
(8) Waddell: Ibid., 1916, viii, 551.

VASECTOMY ON DOGS

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Received for publication June 21, 1924

In a recent paper (12), it was pointed out that vasectomy of itself does not cause degeneration of the germinal epithelium. Most of the discrepancies in the literature regarding this point have been due to failure to control the location of the testes following the operation. A few of the discrepancies appear to have been produced by confinement of the animals or by their diet. It was especially noted (p. 437) that all workers, excepting one, who had used carnivora, reported that no testicular changes result from vasectomy. The exception seemed accounted for in the author's description of his controls but the point appeared worthy of the effort of checking it on the same species of animal. Since the publication of the above report, it has been shown by the writer in coöperation with C. R. Moore that vasectomy on sheep for a period of three months does not cause degeneration of seminiferous tubules (11). It is here intended to present in some detail the results obtained from a series of vasectomy experiments on dogs and to carefully review the literature bearing on this point in animals with testes in a scrotum with the inguinal canal closed. It appears conclusive from these experiments and from the literature that vasectomy does not cause degeneration of germinal epithelium or hypertrophy of interstitial tissue.

Material and technique. Dogs have been used in these experiments first, in order to check the above noted exception, and secondly, in order to gather further information relative to vasectomy on animals with the inguinal canal closed, as in man. The dogs used were picked at random from the laboratory stock and represent the normal run of city dogs. They varied in age from about six months to adults of approximately three years. During the period of experimentation one dog was confined in an animal cage, two were kept in a large room together with other dogs; while five of them lived normal lives. The latter were kept in homes as pets and allowed to run freely in the streets. Controls were selected both from animals confined and from others living normal lives.

All operations were done aseptically under ether anesthesia. The hair was clipped from a region overlying the inguinal canal. This area was shaved and then washed with an alcoholic solution of iodine. A small

incision was then made through the skin and underlying fat; thereby exposing the inguinal canal. A small slit was made through the tunica vaginalis. Through this opening the vas deferens was carefully pulled. It was ligated in two places and a piece about one-half inch in length was excised from between the ligatures. Care was taken not to injure the small blood vessels accompanying the vas. The incisions were closed by interrupted stitches and the dog bandaged in such a manner as to prevent access to the wound. Healing was usually rapid. Observations covered the entire period from the day of operation until the testes were taken for study. In no single instance had the vas deferens regenerated when the testes were removed and all operated tissues showed good recovery.

In order to secure thorough and rapid fixation, the testes were carefully cut into pieces about three-sixteenths of an inch in thickness with a razor blade. These pieces were then immersed in Bouin's fluid. Pieces of these tissues were dehydrated and imbedded in paraffin in the usual manner. Sections cut 7μ in thickness were stained in combinations of Delafield's hematoxylin and Congo red, Delafield's hematoxylin and eosin and Delafield's hematoxylin and orange G. All comparative observations were made upon representative sections taken from corresponding regions of the different testes.

Experimental data: Dog 7. About one year old. Left vas deferens ligated two months during which time the animal was confined in a laboratory cage. The left (ligated) epididymis was somewhat harder and larger than that of the other testis. Microscopic examination showed an exact likeness of tissues in the right and left testes. Spermatogenesis was in abeyance in both testes—no spermatids or spermatazoa were anywhere seen. Evidently confinement had caused discontinuation of spermatogenesis and since the control tissue is here abnormal and both testes are alike in this respect, no conclusions can be drawn from them concerning the effects of ligation.

Dog 8. About six months old. The left vas deferens was ligated two months. During this time the animal was allowed to run freely in the streets. The left epididymis was a little larger and harder than that of the right (unoperated) side. Prepared tissues showed both testes normal and active.

Dog. 14. An adult animal. The left vas deferens was ligated sixty-five days. The dog was kept by some boys and during the last two weeks of this period was actively pursuing a female dog. When the testes were removed they were indistinguishable, both macro- and microscopically.

Dog. 15. An adult animal. The left vas deferens was ligated two months during which time the animal was confined with other dogs in a large room. The epididymis on the left (ligated) side was at first larger and harder than that of the right side, but when the testes were removed they were alike and normal.

Dog. 18. About one year old. The left vas deferens was ligated thirty-nine days. The right testis was cryptorchid. This dog was used as a "teaser" and was very active sexually. Prepared sections of the left (ligated) testis were normal and had an abundance of spermatozoa.

Dog. 23. This dog, an adult, was confined in the dog room during the experiment. The right vas deferens was ligated. The left testis was in the inguinal canal. After

two and one-half months, the right testis (vasectomized) was normal and contained actively dividing germ cells.

Dog. 29. About eight months old. Both vasa deferentia were ligated forty-nine days during which time the dog followed a wagon about the city. The epididymi were hardened and enlarged throughout this period. Both testes presented normal microscopic pictures.

Dog. 30. An adult animal with the left vas deferens ligated two months and five days. The dog, a household pet, lived a normal free life. When castrated, the two testes were indistinguishable macro- and microscopically. The seminiferous tubules were full of both mature and maturing cells.

In none of the above experiments has any change whatever been found in the interstitial cells. It is also of interest to note that each cycle of spermatozoa pushing into the lumen of the tubules carries before it a large mass of debris. This shapeless mass of colloidal material forms a large share of the testicular product which, by its accumulation, causes distention of the epididymis following vasectomy.

Discussion. These experiments clearly indicate that vasectomy in dogs does not cause degenerative changes of the germinal epithelium. Following closure of the vas deferens, there is an accumulation of testicular products in the epididymis which leads to its distention. This is readily noted, in most experiments, as an enlargement and hardening of the epididymis. With special sexual activity, it is conceivable that enough testicular material might be accumulated to cause considerable pressure in the epididymis and perhaps in rare cases to produce some tension within the seminiferous tubules. This material liquefies and is absorbed into the vascular system. An equilibrium between rate of spermatogenesis and absorption of this material is quickly reached. Though spermatogenetic material has been rapidly and abundantly produced in the testes of dogs 14 and 18, absorption has taken place with sufficient rapidity to prevent an excessive pressure within the tubules. The seminiferous tubules of dog testes normally contain a considerable amount of these products but following ligation, these noticeably increase. In none of the above experiments has there been an accumulation of this material sufficient to produce any effect upon the cells of the seminiferous tubules.

The writer wishes to emphasize the fact that post-mortem examination in every case showed that the vas deferens was completely occluded and that there was no escape of products from the vas deferens or from the epididymis. It should be kept in mind that five of the dogs in the above experiments lived natural, free lives and had a diet normal for such animals. In two other experiments, the confinement seems not to have been such as to interfere with the animal's physical well-being. But dog 7, was closely penned up and the conditions found are not to be looked upon as resulting from vasectomy. The literature indicates that such points are worthy of attention. Branca (1) has shown that testes of monkeys in

captivity undergo degeneration even to the point where Sertoli cells alone remain in the tubules. Hartman (7) has shown that in the opossum, failure to ovulate may be due to lack of exercise—confinement. On the other hand Evans and Bishop (4), among others, have shown the importance of diet in the maintenance of spermatogenesis. These points must be taken into consideration in testicular experiments of any kind.

In the above experiments the intertubular tissue has not been affected following two and one-half months of vasectomy. In a recent paper (13) the writer has shown that interstitial cell hypertrophy compensates for a decrease in the size of the seminiferous tubules. Since no tubular degeneration has been found in these experiments, it is natural to find the interstitial cells normal in quantity and in appearance. This further serves to emphasize the fact that changes in the interstitial cell mass are of pressure or tension origin and not of a trophic importance. Spermatogenesis is sometimes slowed up, as in dog 7. If the interstitial cells have a trophic function, there should then be changes in their quantity or size, or at least there should be definite changes in their granular content. No such changes have been found. But in other experiments, given in the paper cited above and in still others not yet reported, the changes in interstitial cell mass have very closely followed variations in the size of the seminiferous tubules where these variations have not been compensated for in the size of the entire testis.

A review of the literature strikingly emphasizes the above points. Hunter (8) in the dissection of a human subject, found the testicle normally developed and containing spermatozoa in spite of the fact that it had no joined vas deferens. Cooper (2) performed a unilateral vasectomy on a dog. Six years later the epididymis was distended with spermatozoa retained by the occlusion of the vas. Shattock, in a microscopic examination of this testis, found the tubuli normal in size and full of epithelial cells with considerable numbers of spermatozoa. Gosselin (5) in a dissection of a man about twenty years of age, found the vas wanting on the right side from the epididymis to the upper part of the bladder. The epididymis was distended with yellow fluid containing a large quantity of dead spermatozoa. In 1853 he vasectomized two dogs and found the testes normal six and ten months later. Curling (3) cited many cases of vas deferens closure and absence with the testicle sound. He concluded that, "The absence or imperfection of the excretory duct does not prevent the development of the testicle at the proper period and has no direct influence in causing it to waste." In each of four experiments (three on dogs and one on a kitten) he found spermatozoa abundant after vasectomy of two to eight months' duration. The epididymi were distended and hardened by the retained fluid. Spangaro (17) found spermatozoa in the testicles of three men, aged seventy, sixty-four and sixty-nine

years, on whom respectively vasectomy had been performed twelve days, six months and two and one-half years previously. The epididymi were distended with spermatic fluid, but the desired degeneration was absent. Shattock and Seligman (14) performed vasectomy on sheep. They found that the epididymis was notably larger than normal from over-distention with the secretion transmitted from the body of the gland and that the tubuli of the testes were filled with epithelial cells and in nearly every one (tubuli) spermatogenesis was in progress. Wallace (18) concluded from vasectomy experiments on dogs that though there is some distention of the epididymis, there is no dilation of the tubules and that the growth of the testicle and its function of producing spermatozoa is independent of the integrity of its vas. He vasectomized six dogs and one kitten for periods of five to twelve months. The epididymi became distended and enlarged by the retention in it of fluid, but in every case the seminiferous tubules were normal in size and full of epithelial cells. In all but two of the dogs and in the kitten, spermatogenesis was active. In these three there was no degeneration and the tubules were full of epithelial cells. A communication to Wallace from A. H. Greg bore the information that the latter had found the testis perfectly normal four years after accidental section of the vas in a radical cure for hernia. Simonds (16) examined a series of cases (human) in which one or both of the vasa deferentia had been closed by cicatrix formation. In thirty cases, spermatogenesis was normal, while in two, the interstitial cells showed proliferation. Unfortunately nothing is known regarding the complication of these two cases that might well account for the fact that they were unlike the other thirty. Wheelon (19) reported that in two unilateral vasectomy experiments of nine months' duration on dogs, "The seminiferous tubules, although showing the results of destructive processes, contain much spermatic tissue. In many of the tubules sperm heads were readily identified; in places spermatids were seen." He thought that there was some interstitial cell hypertrophy. Moore and Oslund (11) vasectomized two sheep seventy-six and ninety days. In both cases spermatogenesis continued and spermatozoa were abundant. In one experiment the epididymis was noticeably larger than in the control and in some of the tubules near the straight (excurrent duct) tubules, the lumen was either obscured or filled with debris consisting of degenerating cells and broken fragments of spermatozoa. The interstitial tissue in both experiments was normal. Kuntz (9), (10) in two series of experiments, each on dogs, reported that ligature and resection of the ductus deferens was followed by degeneration of seminal epithelium and accompanying hypertrophy of the interstitial secretory tissue in both testes. In the last paper he reported that the dogs were confined in relatively close quarters during the experiments and that the control animals had de-

generated testes. These experiments are similar to that of dog 7 of the present series and little can be concluded from them regarding vasectomy. He reports that, "In one dog which was allowed to live one hundred and forty-two days following ligation and resection of the ductus deferens the seminal epithelium was restored." This conclusion was based upon what he thought was an interstitial hyperplasia.

If we omit these last experiments from our review, as examples of confinement rather than of vasectomy as in dog 7 of this series, there are found no exceptions to the general findings that ligation of the vas deferens does not cause degeneration of the germinal epithelium in those animals where the testes are contained in a scrotal sac with the inguinal canal closed. The writer does not contend that no seminiferous tubules whatever showed degeneration, for it is conceivable that a few tubules near the straight (excurrent duct) tubules might, during a period of adjustment, contain enough debris to cause some pressure atrophy. But to argue that such an occasional tubule represents the state of affairs in the testicle is preposterous. A testis with ligated duct from one animal should not only be compared with a control from another animal, but should be compared with the contralateral, uninjured testis of the same animal. Testicular tissue very quickly responds to changes in the physiological state of the animal and it is a common occurrence to find tubules in testes of laboratory dogs in which spermatogenesis is in abeyance. By comparing the vasectomized testis with the unoperated testis of the same animal, such sources of error are eliminated.

It does not appear at all probable that vasectomy causes hypertrophy of interstitial cells. Only in Wheelon's experiments (excepting those of Kuntz) is there recorded interstitial cell hypertrophy and in them it was recorded along with spermatogenesis. The evidence that interstitial cell hypertrophy is a response to change in pressure and tension within the testis following dilatation or atrophy of the seminiferous tubules, seems quite conclusive. Wheelon's two experiments appear to be a doubtful exception to this hypothesis. The writer has never observed interstitial cell hypertrophy resulting from vasectomy. Certainly it has not occurred in the experiments herewith recorded.

The importance of these findings in the field of present day surgery will be evident to the reader. Vasectomy has been advocated as a means of causing degeneration of germinal epithelium and hypertrophy of interstitial cells in order to bring about rejuvenescence. The idea of producing rejuvenescence by ligating the vas deferens sprang from experiments on rabbits, rats and guinea pigs. The writer has, in another paper (12), pointed out that the source of error in those experiments lay in the fact that the changes found were produced not by vasectomy but by artificial cryptorchidism. The evidence obtained from the present series of experi-

ments and from a review of the literature again clearly indicates that vasectomy does not cause degeneration of germinal epithelium or hypertrophy of interstitial cells. It is important to note in this connection that some of the reports herewith reviewed have been upon human tissue actually recovered after occlusion of the ductus deferens.

SUMMARY

1. Following vasectomy, the epididymis is usually distended and hardened by the accumulation in it of testicular products.

2. An equilibrium is quickly reached between rate of production of testicular material and its absorption from the epididymis.

3. The establishment of such an equilibrium is a factor in preventing pressure atrophy in seminiferous tubules.

4. Vasectomy in dogs for a period of two and one-half months has not caused degeneration of germinal epithelium.

5. In all testes, following vasectomy, the interstitial cells were normal, both in quantity and in appearance.

6. Literature reviewed shows that vasectomy on sheep from seventy-six days to one year, on dogs from sixty days to four years, and on man, from six months to four years, has produced no testicular changes, other than distention of the epididymis.

7. The error in the theory of vasectomy as a means of producing rejuvenescence has been pointed out.

BIBLIOGRAPHY

(1) Branca: Compt. rend. Soc. de Biol., 1903, lv, 1034, 1117.
(2) Cooper: Observations on the structure and diseases of the testis. London, 1845.
(3) Curling: On diseases of the testis. Philadelphia, 1866.
(4) Evans and Bishop: Journ. Amer. Med. Assoc., 1923, lxxxi, 889.
(5) Gosselin: Arch. Gen. de Med., 1847, 4 s. xiv, 408.
(6) Gosselin: Ibid., 1853, ii, 256.
(7) Hartman: This Journal, 1924, lxviii, 97.
(8) Hunter: ed. by Palmer, 1841, iv. Philadelphia.
(9) Kuntz: Anat. Rec., 1919, xvii, 221.
(10) Kuntz: Endocrinol., 1921, v, 190.
(11) Moore and Oslund: This Journal, 1924, lxvii, 595.
(12) Oslund: This Journal, 1924, lxvii, 422.
(13) Oslund: Ibid., 1924, lxix, 589.
(14) Shattock and Seligman: Proc. Royal Soc. of London, 1904, lxxiii, 49.
(15) Shattock and Seligman: Trans. Path. Soc. London, 1905, lvi, 57.
(16) Simonds: Deutsch. Med. Wochenschr., 1921, xlvii, 665.
(17) Spangaro: Lo. Sperimentale, 1903, lvii, 243.
(18) Wallace: Trans. Path. Soc. London, 1905, lvi, 80.
(19) Wheflon: Endocrinol., 1921, v, 307.

ON THE RELATION OF LABYRINTHINE AND RETINAL EXCITATIONS IN THE RABBIT

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Received for publication June 14, 1924

When an animal with eyes open is rotated around one of the principal body axes, the compensatory position or nystagmus which results may be due to effects of excitations arising both in the retina and in the internal ear. This was shown by Ewald (1) to be true for the pigeon and by Loeb (2) for the lizard, *Phrynosoma*.

A normal pigeon may show, during rotation, compensatory position or nystagmus, both of head and eyes. If, however, the bird is surrounded by a gray cylinder, no nystagmus, or very little, appears during the rotation but a considerable after-effect appears. If both labyrinths of the pigeon are extirpated, nystagmus is seen during rotation, but no after-effect is observed. When such a pigeon is surrounded by a gray cylinder. there is neither rotation nystagmus nor after-nystagmus.

In the lizard, *Phrynosoma*, labyrinthine excitations may be modified by retinal stimulation. When the eyes of this form are closed and are kept closed during rotation, less rotation-nystagmus appears than when the eyes are open but, on the other hand, there is more after-nystagmus with the eyes closed. A similar reduction occurs if, with the eyes open, a gray-cylinder is rotated with the animal.

In man, optical nystagmus is readily experimentally demonstrable (3), (4). By the rotation in front of rabbits of a moving screen provided with alternate black and white lines, Bartels (3) was unable to demonstrate the presence of optical nystagmus.

The purpose of this communication is twofold; first, to present evidence to show that the response to rotation in the light differs from that in the dark and hence that retinal modification of labyrinthine excitation does occur in the rabbit; and second, that the response to repeated rotation in the light differs from that in the dark.

The mechanical arrangements employed in recording graphically the rotation nystagmus and the after-nystagmus are described in a recent paper by one of us (5). It was also shown that a decrease in the amount of rotation nystagmus occurs after repeated rotation.

In the present investigation young brown rabbits of about 1½ kilograms in weight were used. Each rabbit was given two hundred rotations a day in alternate sets of ten turns to the right and ten to the left. The results are expressed in rotation periods; each period consists of ten turns to the right and ten to the left. The time of each rotation was two seconds. Records were obtained during the first period and during the twentieth. The animals of the first group were rotated in the light; the second were rotated in complete darkness; our method of recording the results permitted the accomplishment of the work in total darkness. Precaution was

TABLE 1

Comparison of the number of eye-movements obtained as a result of rotation of rabbits in the light, with the number obtained in darkness

In the last column, in each group, the sum of the eye-movements, i.e., to the right and to the left, is given.

IN THE LIGHT					IN DARKNESS				
Number of rabbit	Set of rotation	Eye-movements			Number of rabbit	Set of rotation	Eye-movements		
		Right	Left	Total			Right	Left	Total
49	1	54	80	134	47	2	73	61	134
46	1	71	58	129	45	1	42	46	88
34	1	40	48	88	44	1	30	58	88
33	1	62	63	125	43	1	35	51	86
32	1	42	48	90	42	1	50	65	115
30	1	72	60	132	40	1	23	53	76
25	1	71	68	139	39	1	37	61	98
24	2	67	67	134	38	1	47	60	107
21	2	49	62	111	37	1	46	52	98
1	1	78	76	154	35	1	29	46	75
3	2	86	91	177	26	1	49	49	98
5	1	84	84	168	23	2	41	50	91

taken that the animals of the second group did not become totally dark-adapted; this was accomplished by the occasional illumination of the room for periods of five minutes or more. Between successive practice rotations, rests of thirty to sixty seconds were given.

In table 1, the response to the turning in the light during the first rotation period is compared with that in the darkness. The total amount of response in the light is greater than the total in the darkness. That the difference in the responses is due to the effect of retinal stimulation seems to be a reasonable assumption for it is in accord with the fact found by Loeb in the behavior of *Phrynosoma*, rotated with its eyes opened and closed, namely, that the reaction is equal to the algebraic sum of the retinal and the labyrinthine stimulations. In the darkness, therefore,

since only the labyrinth is excited, the reactions would be less than in the light.

Table 2 gives a comparison of the results obtained during the first period of the animals rotated to the right and to the left and during the twentieth.

TABLE 2

Comparison of the reductions of the rotation-nystagmus in the light and in the darkness during twenty rotations

IN THE LIGHT							IN DARKNESS						
Number of rabbit	Set of rotation	Eye-movements		Reduction		Average reduction	Number of rabbit	Set of rotation	Eye-movements		Reduction		Average reduction
		To right	To left	To right	To left				To right	To left	To right	To left	
				per cent	*per cent*	*per cent*					*per cent*	*per cent*	*per cent*
1	1	78	76	58	30	44	38	1	47	60	66	63	65
	24	33	53					20	16	22			
2	1	97	87	45	38	42	37	1	46	52	67	65	66
	26	53	54					20	15	18			
4	1	97	90	39	23	31	35	1	29		65		65
	21	59	69					20	10				
5	1	84	84	23	15	20	26	1	49	49	63	59	61
	20	65	70					22	18	20			
30	1	72		38		38	39	1	37	61	57	57	57
	20	45						20	16	26			
31	1		47		49	49	40	1	23	53	74	53	64
	20		24					20	6	25			
32	1	42	48	40	31	36	42	1	50	65	62	71	67
	20	25	33					20	19	19			
33	1	62	63	65	49	57	43	1	35	51	60	58	59
	20	22	32					20	14	21			
34	1	40	48	58	21	40	44	1	30	58	47	62	55
	20	17	38					20	16	22			

The reduction in the twenty periods of rotation in the darkness is greater than the reduction in twenty periods of rotation in the light. This difference is explicable on the basis of the studies of Pari (6) and Merzbacher (7) and Maxwell, Burke and Reston (8) who have arrived at similar con-

clusions, namely, that with an increase in the intensity of the stimulus there is an increase of the reflex end-effect. The animals rotated in the light may be considered as subjected to a more intense stimulus than those in the dark; in the light the stimulus consists of the excitations both from the labyrinth and the retina while in the dark the stimulus is entirely due to the excitations arising from the labyrinth. As a consequence not only the response to the first, but likewise the response to the twentieth set of rotations would be greater than the responses during similar periods in the dark.

BIBLIOGRAPHY

(1) EWALD: Wiesbaden, 1892.
(2) LOEB: Pflüger's Arch., 1907, cxvi, 368.
(3) BARTELS: Graefe's Arch. f. Opth., 1920, ci, 20.
(4) BORRIES: Acta-Oto Laryngologica, 1923, v, 237.
(5) MAXWELL: This Journal, 1924, lxviii, 125.
(6) PARI: Arch. Ital. de Biol., 1904, xlii, 109.
(7) MERZBACHER: Pflüger's Arch., 1900, lxxxi, 222.
(8) MAXWELL, BURKE AND RESTON: This Journal, 1922, lviii, 432.

DIURESIS IN THE SHEEP

Concentration of Uric Acid and Urea by the Excretory Mechanism of Sheep and Rabbit Compared

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Received for publication June 27, 1924

In a former communication from this laboratory (1) data on uric acid excretion by rodents during diuresis were presented. The variations in amount of uric acid excreted after injection of isotonic and hypertonic salt solutions indicated the non-threshold character of this substance. There was no evidence in the curves of excretion that catabolism in the kidney tissue produced any part of the uric acid eliminated. Rats and rabbits, which are usually credited with blood uric acid content of 2.0 mgm. and 0.05 mgm. and uricolytic indices of 95 and 96, were the experimental animals. Search for an animal exhibiting a different blood-urine relationship led to the ungulates, which are characterized by lower blood uric acid and (according to Hunter and Givens) (2) smaller uricolytic index. Of this order the sheep is accepted as the most exaggerated example of low blood and high urinary uric acid, the analyses of Folin and Denis (3) and Pucher (4) being in agreement on 0.05 mgm. uric acid per 100 grams blood, while Hunter and Givens (2) assigned 80 as its uricolytic index. We believed therefore that use of the sheep as subject for further work offered opportunity to study uric acid excretion under conditions which contrast most markedly with those of the rodents. The results to be presented in this paper are an interesting addition to those previously published (1). Comparison of the kidney function of ungulates and rodents during diuresis, which is made possible, emphasizes the marked similarity of excretory activity of the two groups of animals, though there are points of difference which seem to characterize the two animal orders.

Methods. Our observations were made on a female sheep, weight 44 kgm., fed on oats and hay. The animal was tied down for each experiment and urine collected through a glass tube applied externally and held in place by means of tape and hemostats fastened to the animal's wool. The urine specimens were kept in consecutive order for analysis, each

being identified with its time of micturition. Injection of salt solution was by gravity into the jugular vein.

In the analysis of the urines, uric acid was determined by the method of Morris and Macleod (5). The method of Folin and Wu (6) was used for

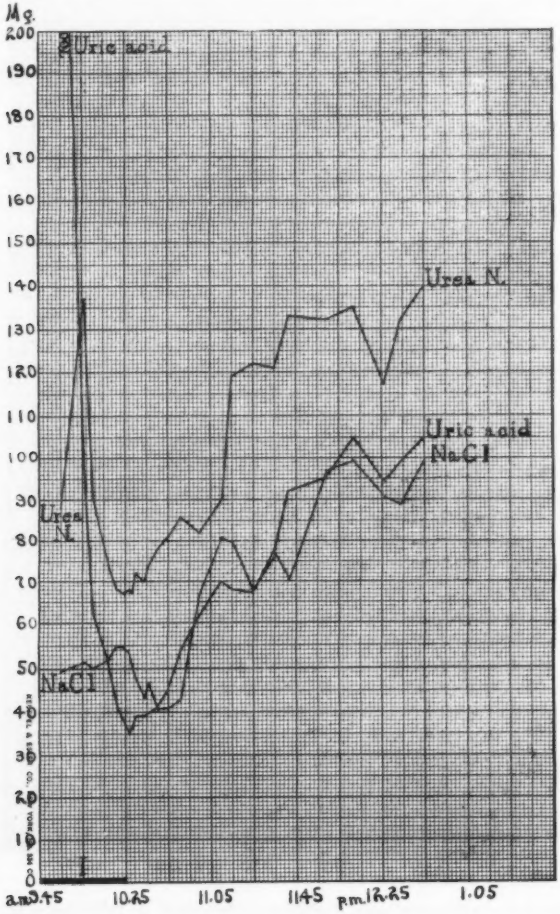

Fig. 1. Isotonic sodium chloride (3100 cc.) injection, *I*. Urea N expressed in milligrams per 100 cc., uric acid values in milligrams per 10,000 cc., sodium chloride in milligrams per 10 cc. to facilitate comparison.

urea nitrogen values. Chlorides were determined by the Whitehorn (7) method.

Experimental. As in the work on rodents, sodium chloride and sodium sulphate were the salt solutions injected. In each experiment isotonic solution was first injected, 50 to 75 cc. per kilogram body weight. Hyper-

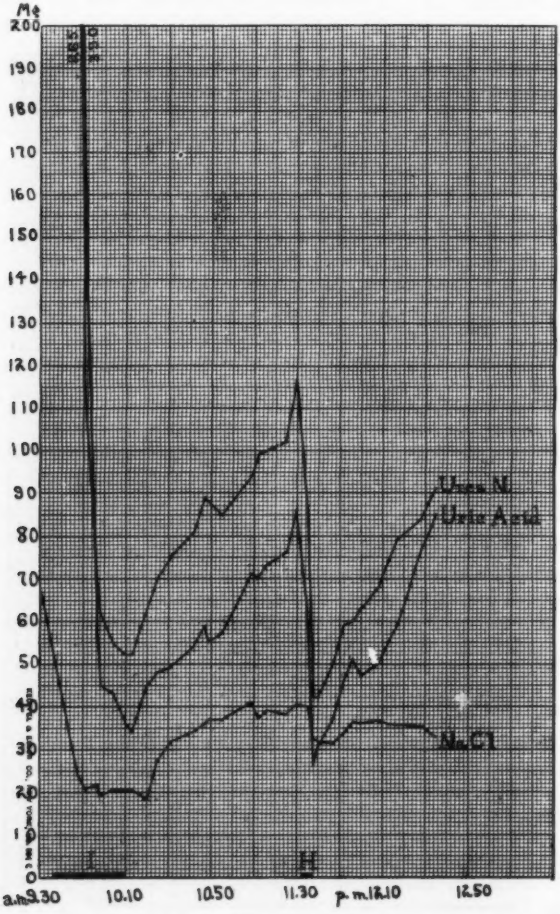

Fig. 2. Isotonic sodium sulphate (2550 cc.) injection, *I*, followed after 1½ hours by (200 cc.) injection of hypertonic sodium sulphate, H. Urea N expressed in milligrams per 100 cc., uric acid values in milligrams per 10,000 cc., sodium chloride in milligrams per 10 cc. to facilitate comparison.

tonic solution of the same salt (5 times isotonic) was injected two hours later, in about one-tenth the volume of the isotonic. Figures 1, 2, 3 represent the excretion of urea, uric acid and chlorides, the analytical

results being calculated on a concentration basis. In figure 4 appear the urea and uric acid curves following administration of 60 grains of atophan by stomach tube.

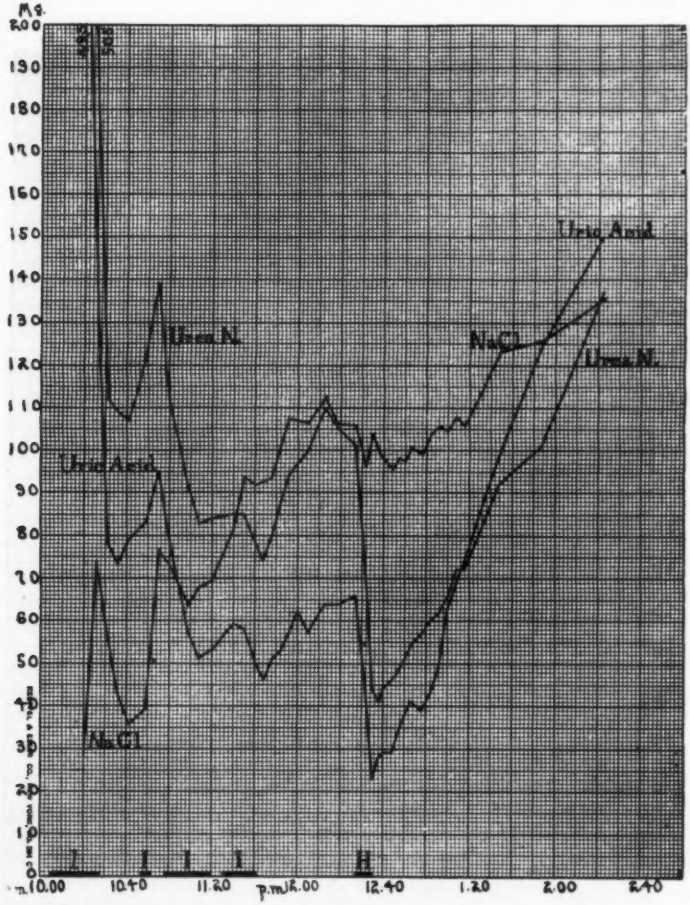

Fig. 3. Isotonic sodium chloride in interrupted injection, *I*, *I*, *I*, *I*, (total 2950 cc.), followed after 1 hour by (400 cc.) injection of hypertonic sodium chloride, H. Urea N expressed in milligrams per 100 cc., uric acid values in milligrams per 10,000 cc., sodium chloride in milligrams per 10 cc. to facilitate comparison.

Discussion. The previously noted parallelism between urea and uric acid excretion is confirmed in all the present series. Variations in the amounts of both substances depend upon the same factors, of which the

volume of urine eliminated is certainly of great importance. It is especially interesting to note that in the atophan series (fig. 4) this parallel excretion is equally marked, though the concentration of both urea and uric acid is ten times that of diuresis.

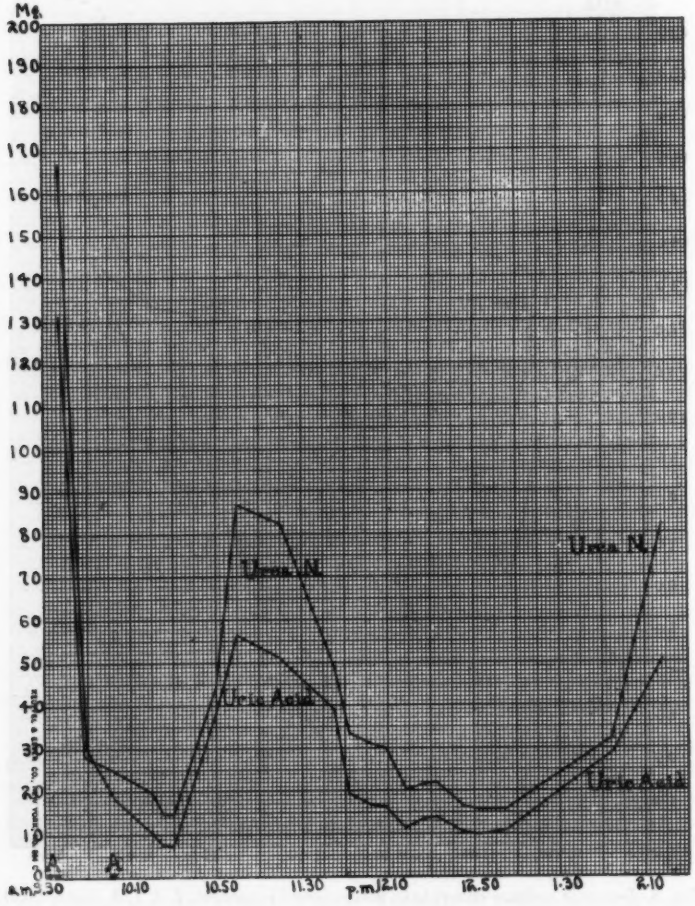

Fig. 4. Atophan given by stomach tube (30 gr. + 30 gr.), *A*, *A*. Urea N expressed in milligrams per 10 cc., uric acid values in milligrams per 1000 cc. to facilitate comparison.

In an effort to gain more knowledge of the kidney mechanism for the elimination of these waste products, we considered it advisable to calculate the degree of concentration effected in the transfer from blood to

urine in each of the sheep series. Before doing so, however, we thought it best to analyze sheep blood for urea and uric acid, since available values were obtained several years ago with methods that have been often improved or replaced by methods which are much more reliable. The experimental sheep, by the Folin-Wu method, showed a urea N value of 24 mgm. per 100 cc. and abattoir sheep blood agreed closely with 23 mgm. per 100 cc. Uric acid analysis was carried out on 1000 cc. of tungstate filtrate (corresponding to 100 cc. blood) by a modification of the Morris and Macleod method. The usual precipitation as zinc urate was carried out on each of twenty 50 cc. quantities, each precipitate dissolved in 10 per cent hydrochloric acid and then all combined into one specimen for colorimetric determination. An excess of 10 per cent sodium cyanide (15 cc.) was added in order to dissolve the precipitate of zinc cyanide before addition of arseno-18-tungstate reagent. To make the standard comparable, 20 cc. of 2.5 per cent zinc chloride and 15 cc. 10 per cent sodium cyanide were added. The experimental sheep had a blood uric acid of 0.18 mgm. per 100 cc. and abattoir sheep blood 0.29 mgm. per 100 cc. (the stated quantities being thus actually determined in the analysis and not calculated). Though the values secured are considerably higher than earlier analyses indicated was present, we believe they represent more nearly the actual quantities. Using the figures 24 mgm. and 0.18 mgm. for blood urea N and uric acid, table 1 represents the extent to which these substances were concentrated by the kidney at the beginning, during and at the close of each experiment. During the diuresis extreme conditions were chosen, such as the lowest concentration and highest volume following both isotonic and hypertonic injections.

TABLE 1

Relative concentration of urea and uric acid in sheep urine and blood (conc. in blood taken as unity)

	FIRST URINE		LOWEST CONCENTRATION PER CUBIC CENTIMETER AFTER ISOTONIC INJECTION		PEAK OF DIURESIS AFTER ISOTONIC INJECTION		LOWEST CONCENTRATION PER CUBIC CENTIMETER AFTER HYPERTONIC INJECTION		PEAK OF DIURESIS AFTER HYPERTONIC INJECTION		LAST URINE	
	Urea	Uric acid	Urea	Uric acid	Urea	Uric acid	Urea	Uric acid	Urea	Uric acid	Urea	Uric acid
Sodium chloride.....	37	59	2.8	2.0	3.1	2.2					5.8	5.8
Sodium chloride.....	21	27	3.1	2.5	3.2	2.8	1.7	1.3	1.8	1.7	5.7	8.3
Sodium sulfate	68	114	2.2	1.9	3.7	3.1	1.7	1.4	1.7	1.7	3.8	4.7
Atophan.....	54	92	6.0	4.2							34.0	27.8

It is evident that there is great similarity between the figures for chloride and sulfate series. At the times of greatest concentration the uric acid ratio is larger than that of urea, as for example at the start of each series, while during diuresis the uric acid ratio is somewhat lower than the corresponding urea ratio. Very evidently variations in elimination of the two substances are quite similar in kind. Such constancy of excretion, in spite of marked differences in chemical structure and physical properties (e.g., solubility), must indicate the functioning of a common mechanism for the two substances.

In order to compare this picture of ungulate excretion with that of rodents, there are presented in table 2 figures for urine-blood concentra-

TABLE 2

Relative concentration of urea and uric acid in rabbit urine and blood (conc. in blood taken as unity)*

20 MINUTE PERIOD	RABBIT 4		RABBIT 5		RABBIT 6	
	Urea	Uric acid	Urea	Uric acid	Urea	Uric acid
1	2.3	13.6	1.9	2.4	5.2	7.3
2	1.3	5.3	1.5	1.9	7.2	12.1
3	1.7	7.3	2.0	2.7		
4	2.1	8.1			4.0	5.7
5	2.3	8.4	4.0	10.7	2.8	3.9
6	2.6	7.6			1.9	2.4
7	1.4	2.5	2.7	4.0	1.8	2.3
8	1.5	2.4	1.8	2.0	1.2	1.5
9	1.9	3.6	2.6	3.6	2.1	3.6
10	2.7	7.2	4.3	7.0	2.4	5.7
11	3.7	9.6	5.5	9.6	2.8	8.0
12	4.4	11.3			3.2	10.1

* Based on Morris and Rees (1) analyses.

tion ratios of rabbits. The urine analytical data are those secured in this laboratory by Morris and Rees (1). The blood values (31 mgm. urea N and 0.75 mgm. uric acid) were analytical results obtained by the present authors on blood taken from the heart of a normal rabbit. Urea N was determined by the Folin-Wu method and uric acid as indicated above for sheep blood (550 cc. filtrate being precipitated with zinc and the precipitates combined for one determination). Since the rabbits were catheterized and the bladders rinsed with isotonic salt solutions, all the urines must be looked upon as taken during the course of the experiments. The urea ratios of the rabbits should therefore be compared with those of the last sheep urine taken or those during the diuresis, not with the first urines,

which were uncertain mixtures collected at different points in the transition from the normal condition to that of diuresis. So compared, it is obvious that the concentration of urea, during diuresis, proceeds much the same in the rabbit and sheep.

Concentration ratios of uric acid, on the other hand, are consistently larger for the rabbits than for the sheep. This point of difference between the two kinds of animals probably has some relation to the higher level of purine metabolism of rodents. The higher blood uric acid content may be the cause of a greater relative efficiency of the rabbit's mechanism for concentrating uric acid. However it is also possible that the rabbit kidney is adding uric acid through its cellular activity, while the sheep kidney is not capable of making a comparable contribution, or that the sheep kidney destroys uric acid. Whatever may be the explanation, the uniform variation in uric acid concentration during diuresis suggests that different degrees of efficiency of organic function may characterize different animal orders.

SUMMARY

During salt diuresis in the sheep, uric acid and urea excretion are parallel. The urea concentration ratio (urine : blood) of sheep is like that of rabbits, while the uric acid ratio is consistently greater in rabbits. Suggestion is made that the higher level of purine metabolism of rodents may be responsible for an increased kidney efficiency in concentrating uric acid.

BIBLIOGRAPHY

(1) Morris and Rees: This Journal, 1923, lxvi, 383.
(2) Hunter and Givens: Journ. Biol. Chem., 1914, xviii, 403.
(3) Folin and Denis: Journ. Biol. Chem., 1912, xiv, 29.
(4) Pucher: Journ. Biol. Chem., 1922, lii, 317.
(5) Morris and Macleod: Journ. Biol. Chem., 1922, l, 55.
(6) Folin and Wu: Journ. Biol. Chem., 1919, xxxviii, 81.
(7) Whitehorn: Journ. Biol. Chem., 1921, xlv, 449.

THE TOXICITY OF THE "ACETONE BODIES"[1]

I. Acetone Administered Intravenously

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Received for publication May 24, 1924

The observations which are herewith presented were the outgrowth of a study in which acetone and normal saline were used together as solvents for another drug whose activity was being studied. This unfortunately introduced an additional but unavoidable factor, and therefore a parallel series of experiments was performed using a similar solution of the acetone with normal saline, but no other drug. It is this latter series of controls to determine the pharmacological action of acetone on intravenous injection that is reported here.

Acetone is a unique solvent inasmuch as it has many of the properties of the organic solvents, but differs from most of them in being miscible with water in all proportions. It would, therefore, be an excellent vehicle for certain drugs not soluble in water whose action on intravenous injection it is desired to study. The action of acetone is also important because it is so frequently a constituent of the body in disease. It is well known that a close relationship of the "acetone bodies" exists in diabetes, acid-intoxication and acetonuria. Later reports will be made on the comparative toxicity of acetone, diacetic acid, dextro and levo beta oxybutyric acid, racemic beta oxybutyric acid, etc.

Historical. An examination of the literature reveals a vast collection of clinical material on the interplay of the acetone bodies in diabetes mellitus, starvation and many infectious diseases (1). The experimental data on animals, however, are rather limited, and especially those referring to the continuous introduction of acetone or solutions of acetone directly into the blood stream, where the effective concentration can be quickly reached. An overwhelming single dose ought not be given. Wilder (2), by means of a specially devised volumetric pump, made continuous intravenous

[1] A preliminary communication of this work was given at the Cincinnati meeting, December, 1922, of the American Society for Pharmacology and Experimental Therapeutics. The unfortunate drowning of Doctor Seybold has robbed us of his fine-spirited coöperation and criticism.

injections of the sodium salts of beta hydroxybutyric and acetoacetic acids in dogs. In the concentrations used by him, 0.0017 to 0.0083 gram per kilogram per minute, no toxic effects were reported. He was interested in the intravenous tolerance limit or overflow level into the urine and the type of acetone body eliminated in the urine. Marriott's recent work is quoted below. Acetone, when given by mouth, per rectum or subcutaneously, has been shown by Widmark (3) to be non-toxic in rather large doses, and does not attain high concentration in the blood stream either early or late. Next to the intravenous route the most rapid absorption of acetone has been shown to take place in rabbits by intraperitoneal injection. In 1884, Albertoni (4) reported a few experiments in dogs by the intravenous administration of 20 to 33 per cent acetone. These doses were toxic but no death ensued. The acetone concentration in the blood was approximately 1.27 to 1.89 per cent by calculation. In 1892, Roque, Devic and Hugouneucq (5) injected diabetic blood intravenously into rabbits. They complicated their problem by the use of a foreign protein and therefore their results cannot be considered. The experiments on rabbits performed by Archangelsky (6) are of great interest. He determined the blood acetone concentration while subjecting the animals to air containing acetone. Narcosis was present when the blood held 0.32 to 0.83 per cent acetone. Kussmaul was the first to show experimentally, in 1874, that acetone injected subcutaneously in rabbits had an anesthetic effect. In 1910 Cushny (7) reported three experiments on tracheotomized cats anesthetized with ethyl urethane and given acetone intravenously in doses ranging from 0.034 to 0.8 gram per kilogram. No toxic effects were noted by him. Widmark (3) reported a series of experiments on rabbits in which the blood-acetone concentration was determined after administration of acetone per os, subcutaneously, per rectum, intraperitoneally and intravenously. He reports no toxic effects in the rabbits even when the acetone was present in the blood up to 0.8 per cent. More recently, Briggs and Shaffer (8) in studying the excretion of acetone from the lungs administered 1.0 to 1.45 grams of acetone per kilogram intravenously in dogs narcotized with urethane. They noted no toxic effects.

Experimental part. A 50 per cent solution of purified acetone in normal saline (0.9 per cent) was administered intravenously and continuously from a burette or Woodyatt apparatus (9) in etherized and non-etherized dogs, cats, rabbits and guinea pigs. In those animals not given ether, operative work was done after local administration of cocaine. The figures given for acetone are on the basis of pure acetone, and not the 50 per cent solution. The rate of injection varied from 0.045 to 1.190 cc. of acetone per kilogram of body weight per minute in dogs. In the cats, rabbits and guinea pigs the rate of injection was 0.319 to 3.430 cc. per

kilogram per minute. Effects on respiration, blood pressure, pulse rate and regularity, and the clotting of blood were noted. No organs were isolated. In one experiment the right vagus was ligated, and in another atropine was injected intravenously.

It will be noted that all of our blood-acetone concentrations are arrived at by calculation. There are several reasons for not carrying the work out with chemical analyses. First, because there was no reason to do so in the original situation where our interest was centered in the toxicity of a certain drug dissolved by the acetone solution rather than in the acetone itself. Reporting of the acetone control experiments was really an afterthought and we felt desirable because we could find very little information on the toxicity of acetone administered intravenously. It will also be

TABLE 1

NUMBER OF EXPERIMENTS	WEIGHT	LETHAL DOSE PER KILOGRAM BODY WEIGHT	AMOUNT INJECTED PER MINUTE	TIME INJECTION TO DEATH	BLOOD 9.67 PER CENT OF BODY WEIGHT	BLOOD VOLUME WEIGHT ÷ 1.050	BLOOD ACETONE PER CENT PER MINUTE INCREASE	ACETONE PER CENT IN BLOOD AT DEATH	RATE PER KILOGRAM PER MINUTE
	kgm.	*cc.*	*cc.*	*minutes*	*grams*				*cc.*
Dog 1	5.6	1.50	6.67	*1.26*	538	512	*1.303*	1.64	*1.190*
Dog 2	8.6	4.66	4.64	10.41	825	786	0.593	4.29	0.539
Dog 3	12.5	5.18	1.33	*48.50*	1,200	1,143	0.116	5.67	0.107
Dog 4	8.0	6.00	4.05	11.85	768	731	0.554	*6.60*	0.506
Dog 5	11.2	3.39	2.80	13.95	1,075	1,024	0.273	3.53	0.250
Dog 6	6.0	1.04	0.43	14.50	576	548	0.078	1.14	0.072
Dog 7	*4.5*	0.77	0.60	5.83	432	411	0.146	*0.85*	0.133
Dog 8	6.5	2.65	0.54	31.75	624	594	0.099	2.80	0.083
Dog 9	10.5	1.09	0.47	24.50	1,008	960	*0.049*	1.19	*0.045*
Dog 10	*12.5*	2.72	1.94	17.50	1,200	1,143	0.169	2.97	0.155

The italicized figures are the maximum and minimum for the respective column.

noticed below in our reference to Widmark's work that he has shown that acetone disappears very slowly from the organism even after one hour, therefore a calculated figure would probably be quite close to the actual figure as determined by chemical analysis. Further progress in the "acetone phase" of this study was interrupted but we have been advised that a report of the results ought not to be delayed any longer.

Experimental data: Dogs. Ten experiments were performed on dogs and a summary of some of the essential data is embodied in table 1.

The weights of the animals ranged from 4.5 to 12.5 kgm., and the acetone was injected at rates varying from 0.045 to 1.190 cc. per kilogram per minute. The total blood volume of a dog is on an average 9.67 per cent of the body weight (10); and after correcting for specific gravity to get blood volume it was possible to calculate the percentage of acetone

present in the total blood volume per minute. This percentage varied from 0.049 to 1.303 on the basis of no excretion or destruction. The experiments lasted from 1.26 to 48.5 minutes before the animal expired, and at the terminal stage there was estimated to be 0.85 to 6.60 per cent of acetone in the blood if none of the acetone had been eliminated or removed which, of course, is not probable. Widmark (3) has shown the relationship of the invasion factor to the evasion factor of acetone in the blood, that is, the injection rate as compared to the removal rate. His experiments performed on rabbits and man demonstrate the relative importance of the factors affecting the fall in concentration in the blood. The factors considered by him were *a*, elimination through the lungs; *b*, elimination through the urine; and *c*, the chemical or intermediary metabolism. Acetone, he says, disappears very slowly from the organism. If a certain quantity of acetone is continuously introduced into the system there must appear after a time an approximate state of equilibrium in which the supply and the loss are equally great. This state of equilibrium is rapidly reached with a relatively low concentration. During the time of administration by vein there was no sharp drop in concentration (exper. 12 of Widmark), although there was some fall in his experiment with the rate of injection 0.025 cc. per kgm. per minute. After the cessation of acetone injection by vein the initial acetone concentration of 0.50 to 0.80 per cent dropped after 60 minutes to approximately 0.40 per cent and remained there for many hours. As a matter of fact, Widmark introduced the acetone in a few moments, while in our experiments the acetone was injected continuously until the terminal stage appeared, therefore equilibrium was very quickly reached. Our calculated figures of the per cent of acetone in the blood at the terminal stage are undoubtedly somewhat high since we have introduced no correction for the excretion or destruction of the acetone.

The toxic effect in the majority of our experiments manifests itself first in an alteration of either respiratory rate or depth, or both. At this time from 5.88 to 57.7 per cent of the lethal dose had been administered. Some of the earliest investigators (Kussmaul, Buhl and others (11)) noted the striking resemblance to chloroform in the respiratory changes of acetone, and in fact acetone was designated as an *indifferent narcotic* many years ago. In experiment 1, where the injection was made rapidly, 1.19 cc. per kgm. per minute, both the respiratory and circulatory mechanisms were affected simultaneously. There was no consistent effect in the experiments as to the rate or depth of respiration. The following grouping was observed, *a*, the respiratory rate and depth both decreased in three experiments; *b*, the rate increased and the depth decreased in one experiment; *c*, the respiratory depth or rate decreased with or without a simultaneous increase in depth with the rate change in four experiments

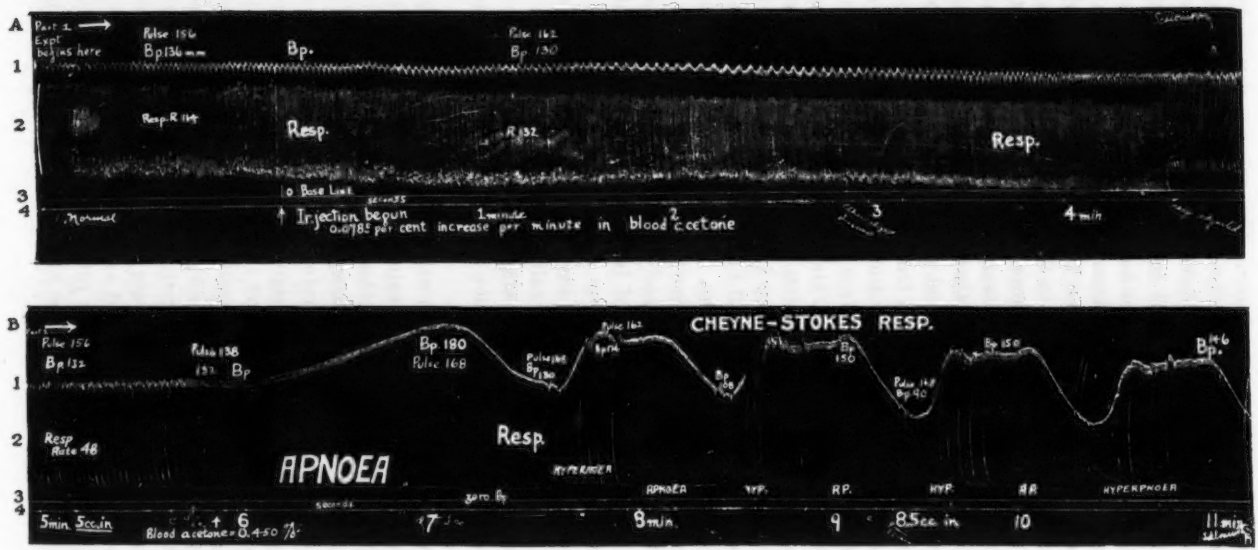

Fig. 1. Reading from the top of these two figures, the first line represents blood pressure, the second line respiration, the third line zero blood pressure, and the fourth line time in seconds.

A: First portion of the experiment, showing the normals. Arrow below seconds line indicates when injection of acetone was started. In B the decrease in respiratory rate is evident with the concomitant changes in the blood pressure and heart rate.

each of which had a low percentage of acetone given intravenously; and *d*, both rate and depth increased in two experiments. In a few experiments after the initial decrease in rate and depth there followed an increase in both. In experiment 3 the right vagus was ligated at the very start and the rate and depth both decreased. In experiment 4, after the administration of atropine the blood pressure dropped temporarily and for an interval of several minutes there were periods of dyspnea which lasted about five seconds each.

There was ultimately respiratory depression and paralysis in practically all the experiments before the terminal fall in blood pressure occurred.

In experiment 6 a most striking example of Cheyne-Stokes respiration occurred. This animal was given acetone at the rate of 0.072 cc. per kilogram per minute or a 0.0785 per cent increase in the acetone content of the blood per minute. In 1.25 minutes after starting the injection, the respiratory rate was increased, and the depth slightly diminished. Then the respiratory rate decreased progressively and later quite a good deal until stoppage of the respiration occurred within 5.75 minutes from the initial administration of acetone. At this time the total blood acetone percentage was approximately 0.450. A period of respiratory paralysis ensued lasting about two minutes during which time the blood pressure rose very gradually (in 1.25 minutes) from 130 mm. Hg to 180 mm. and then dropped within 0.75 minute back to 130 mm. Hg. At this time a 0.40 minute period of hyperpnea ensued, followed by apnea for 0.5 minute. At about the second respiration in the dyspneic period the blood pressure rose again but now rather abruptly up to 170 mm. Hg, remained up for about 0.45 minute and then dropped abruptly to 108 mm. Hg when a second period of hyperpnea developed. This phenomenon was repeated for a number of times, and is shown in figure 1. The heart was regular with the beats at an average of 165 per minute. The literature on the various types of periodic respiration has been reviewed by Eyster (12), Barbour (13) and Hewlett (14).

There is a tendency for the blood pressure to rise somewhat with low concentration and slow injection, and to drop with rather rapid injections of acetone or when the initial blood pressure is high. In some experiments there was very little change in the blood pressure until the heart became irregular; in other words, there was very little effect on the vasomotor center. An initial alteration in the blood pressure occurred when approximately 26.4 to 100.00 per cent of the lethal dose had been administered. In practically all the experiments the heart finally became irregular and rapid. The heart rate then decreased very quickly until stoppage occurred. This took place simultaneously with the fall in blood pressure. Following the death of a number of the animals the hearts were sectioned and very little evidence of extensive clot formation was found.

It appears, therefore, that the vasomotor center is very much less susceptible to acetone than the respiratory center, while the heart in turn is more susceptible than the vasomotor center to acetone.

Cats. In table 2 under A are shown the data derived from three experiments on cats with weights ranging from 0.8 to 2.96 kgm. The acetone was injected at the rate of 0.676 to 1.890 cc. per kilogram per minute, and death occurred in from 1.4 to 3.0 minutes. The blood contained 3.8 to 5.1 per cent of acetone at the time that death occurred. The acetone percentage increase per minute in the blood ranged from 1.29 to 3.63. In the cat as in the dog the respiration was first affected by the acetone.

TABLE 2

NUMBER OF EXPERIMENTS	WEIGHT	LETHAL DOSE PER KILOGRAM BODY WEIGHT	AMOUNT INJECTED PER MINUTE	TIME INJECTION TO DEATH	BLOOD*	BLOOD VOLUME	BLOOD ACETONE PER CENT PER MINUTE INCREASE	ACETONE PER CENT IN BLOOD AT DEATH	RATE PER KILOGRAM PER MINUTE
				A. Cat					
	kgm.	*cc.*	*cc.*	*minutes*	*grams*	*cc.*			*cc.*
1	2.8	2.5	5.30	1.40	154.0	146.0	3.63	5.1	1.890
2	2.96	2.0	2.00	3.00	163.0	155.0	1.29	3.8	0.676
3	0.80	2.5	0.83	2.40	43.2	41.0	2.02	4.9	1.037
				B. Rabbit					
1	1.96	4.0	1.63	4.90	105.8	100.0	1.63	8.0	0.831
2	1.76	4.6	0.71	11.25	95.0	90.0	0.79	8.0	0.403
3	1.72	5.1	0.55	9.08	93.0	88.5	0.62	5.7	0.319
				C. Guinea pig					
1	0.67	1.72	2.30	0.50	28.0	26.6	8.62	4.3	3.430
2	0.69	1.61	0.41	2.67	28.0	26.9	1.52	4.1	0.594
3	0.615	1.62	0.54	1.85	25.0	23.8	2.20	4.2	0.878

*Blood volume in per cent of body weight: cats 5.5; rabbits 5.4; and guinea pigs 4.1.

Rabbits. In table 2 under B are recorded the data from three experiments on rabbits weighing from 1.72 to 1.96 kgm. The acetone was injected at the rate of 0.319 to 0.831 cc. per kilogram per minute, and these doses were toxic in 4.9 to 11.25 minutes. The acetone entered the blood at the rate of 0.621 to 1.63 per cent per minute. At the terminal stage 5.7 to 8.9 per cent of acetone was present in the blood by calculation.

A peculiar phenomenon occurred in the rabbits as well as the guinea pigs that did not appear in the cat or dog. Late in the course of acetone

administration a marked twitching of the hind limbs became evident which finally had the semblance of a running attitude. Storm van Leeuwen (15), in 1914, called attention to this phenomenon in the anesthetization of rabbits with chloroform. He called the movements "Laufbewegungen."

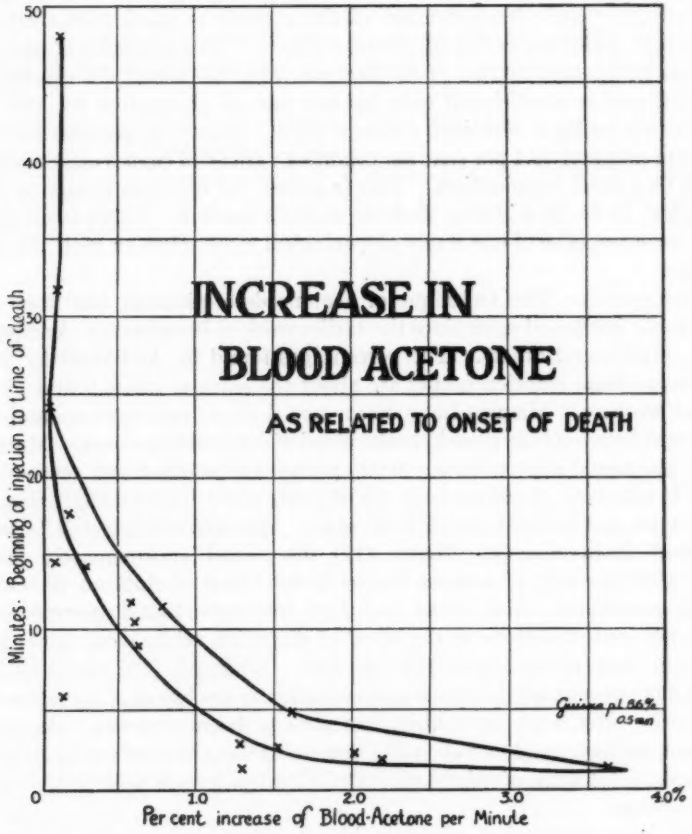

Fig. 2. Ordinate shows time in minutes when death occurred at the concentration indicated in the abscissa. Abscissa gives the rate per minute in the percentage increase of acetone in the blood.

Guinea pigs. Three experiments were performed on guinea pigs with body weights of 0.615 to 0.690 kgm. These are shown in table 2, C. Acetone was injected at the rate of 0.594 to 3.34 cc. per kilogram per minute and the animals lived from 0.5 to 2.67 minutes. The blood acetone percentage increase ranged from 1.52 to 8.60 cc. per minute.

The comparative toxicity of acetone in all the animals irrespective of species is illustrated in figure 2. The dose as related to the time of death produces a fairly consistent curve between two ranges. These two ranges are at the extremes of the curve. When the increase in concentration of acetone in the whole blood volume per minute is high (3.0 per cent) the animals live for a very short period amounting to about two minutes. An effective toxic concentration of the acetone is quickly reached and shown by paralysis of the respiratory center. This paralysis apparently occurs in the first contact of the acetone with the cells of the respiratory center, and is conditioned only by the rate of absorption by the cell before amassing a sufficient concentration. Below an acetone increase of approximately 0.1 per cent per minute a variety of causes can evidently lead to a fatal termination. This is shown by the vast range, in time roughly 10 to 45 minutes, that the animals survive. There is no clean-cut toxic response of one single physiological mechanism as with the high range.

Discussion. The technique of acetone determination has been considerably improved upon since the earlier work of Ruschhaupt, Archangelsky, Müller and others. The percentages found by Archangelsky when pharmacologic reaction occurs are about the same as those found by the other workers. Magnus-Levy discovered 0.16 per cent acetone and 0.22 per cent beta oxybutyric acid in the blood of a diabetic in coma. Marriott (16) has found approximately 0.025 per cent of acetone and aceto-acetic acid in a diabetic, 0.006 per cent in a phloridzinized dog, and approximately 0.019 per cent in a diabetic child in coma. Marriott also injected "acetone bodies" intravenously. Moore (17) discovered approximately 0.0013 to 0.1826 per cent of acetone bodies in the blood of children in various toxic conditions. Van Slyke and Fitz (18) have found approximately 0.25 per cent of acetone in the blood of diabetics, while those under good control show up to about 0.04 per cent. Widmark (19) found as high as 0.334 per cent total acetone concentration in the blood of diabetics with acidosis. His work on rabbits has already been reviewed. As stated above, we have made no chemical determinations, but on the basis of the experimental work of others our calculated figures are close to the actual percentage.

We have shown that when the blood acetone is increased at rates of 0.049 to 8.60 per cent of the total blood volume per minute, toxic responses occur. The essential features of this toxic reaction are similar to those described by Tappeiner (20) and others when using other routes of administration of the acetone.

SUMMARY

1. Acetone is toxic when given intravenously in doses at the rate of 0.045 to 1.303 cc. per kilogram per minute in dogs; and in doses of 0.403 to 3.430 cc. per kilogram per minute in cats, rabbits and guinea pigs. The animals lived from 0.5 to 48.5 minutes.

2. The time that the animal lives is inversely proportional to the size of the dose per minute; particularly when the rate of administration is such as to increase the acetone percentage in the blood 0.1 to 3.0 per minute.

3. Acetone produces depression in the following order: respiratory center, heart and vasomotor center.

The authors take great pleasure in acknowledging the kind criticism and advice of Prof. A. S. Loevenhart, of the University of Wisconsin Medical School.

BIBLIOGRAPHY

(1) Waldvogel: Die Acetonkörper, 1903.
Magnus-Levy: Die Acetonkörper, Ergeb. d. Innere Medizin, i, 1908.
(2) Wilder: Journ. Biol. Chem., 1917, xxxi, 59.
(3) Widmark: Acta. Medica Scand., 1919, lii, 87.
(4) Albertoni: Arch. Exper. Path. u. Pharm., 1884, xviii, 218.
(5) Roque, Devic et Hugouneuco: Rev. de Med. 1892, 995.
(6) Archangelsky: Arch. Exper. Path. u. Pharm., 1901, xlvi, 347.
(7) Cushny: Journ. Physiol., 1910, xl.
(8) Briggs and Shaffer: Journ. Biol. Chem.
(9) Woodyatt: Journ. Biol. Chem., 1917, xxix, 355; 1920, xli, 315.
(10) Meek and Gasser: This Journal, 1918, xlvii, 302.
Whipple, McQuarrie, Davis et al: Ibid., 1920, li, 257.
(11) Kussmaul: Deutsch. Arch. f. Klin. Med., 1874, xiv.
Buhl: Zeitschr. f. Biol., xvi,413.
(12) Eyster: Journ. Exper. Med., 1906, viii, 565.
Eyster, Burrows and Essick: Ibid., 1909, xi, 489.
(13) Barbour: Journ. Pharm. Exper. Therap., 1914, v, 393.
(14) Hewlett: Path. Physiol. Internal Diseases, 1917, p. 353, 394.
(15) Storm van Leeuwen: Pflüger's Arch., 1914, clix, 291.
(16) Marriott: Journ. Biol. Chem., 1913, xvi, 293.
(17) Moore: Amer. Journ. Dis. Children, 1916, xii, 244.
(18) Van Slyke and Fitz: Journ. Biol. Chem., 1917, xxxii, 495.
(19) Widmark: Biochem. Journ., 1919, xiii, 430.
(20) Tappeiner: Deutsch. Arch. f. Klin. Med., 1884, xxxiv, 127.

ELECTRICAL STUDIES IN MAMMALIAN REFLEXES

IV. The Crossed Extension Reflex

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Received for publication June 18, 1924

In the first paper of this series (1) there was described a method of recording spinal reflexes in what seemed to be the most direct possible way, namely, by leading-off monophasic action currents from the motor nerve of a muscle normally involved in the reflex response. We call this method the most direct, since it reduces the reflex arc to its simplest possible terms, involving only nervous tissue, and eliminating from the picture the muscle which is physiologically distinct. That paper dealt with the response of the motor nerve in the flexion reflex to stimulation of an afferent nerve by a single induction shock, in this way using what seems to be the simplest and most regularly obtainable of all the spinal limb reflexes, and simplifying that reflex by evoking it through a single volley of impulses, and thereby avoiding as far as possible the complicating factor of temporal summation. Our results seemed to indicate that the response in the motor nerve consists of impulses in no way differing individually from those obtained by direct stimulation of the same nerve. They appear in an approximately synchronous volley, and yet are much less perfectly synchronized than those recorded from the nerve to which the stimulus was directly applied. The galvanometric records seemed to indicate a dispersion in time of the individual impulses amounting to about 10σ. This dispersion must signify either a difference in conduction time in the individual arcs, or repetitive discharge in some of the motor neurones, or both. There was no definite evidence that in this reflex, evoked by a single shock, the individual motor neurone responds with more than a single impulse, and yet we in no way disproved the possibility that this might be the case. Some experiments by Sherrington (4) have shown that frequently the flexion reflex, even when evoked by a single induction shock, does involve a repetitive discharge in the motor neurones (cf. also (5)). Furthermore, in another paper of this series (3) evidence has been presented indirectly leading to the conclusion that in the absence of spinal transection a considerable measure of after-discharge, consisting, presumably, of repetitive response in some of the motor neurones, is regularly characteristic of the flexion reflex.

It is our object in the present paper to describe the results of an attempt to apply the same general procedure to the study of the crossed extension reflex. If these results appear meager as regards what can be observed by this method, we feel that they are no less significant as showing what cannot be observed.

Salient differences between the flexion reflex and the crossed extension reflex were long ago described by Sherrington (6). Briefly, these are as follows: the flexion reflex is a brisk response, readily evoked by single shocks, having brief latency and relatively transient after-discharge. The crossed extension reflex, on the other hand, is often unobtainable with single shocks, but is highly amenable to temporal summation. The extent of contraction builds up gradually as the stimuli are repeated, and the after-discharge is usually very prolonged. Whereas the former reflex is brisk and transient, the latter is slow and sustained. This sustained character of the crossed extension reflex makes it more nearly resemble, superficially at least, voluntary contraction, which rarely, if ever, so closely approximates the simple neuromuscular twitch as does the flexion reflex when evoked by a single shock.

Liddell and Sherrington (7) have recently made a careful study of the differences between the flexion reflex and the crossed-extension reflex, using a highly refined method of isometric recording of the contraction of the muscles involved. In this study they have clearly shown an even more profound difference between these reflexes than was apparent in Sherrington's earlier work. By comparing the flexion reflex with the myo-neural twitch of the same muscle they conclude that in this reflex at the very beginning of a prolonged series of afferent stimuli all of the motor neurones which take part in the reflex are immediately called into action. In the crossed extension reflex, on the other hand, the first few stimuli of a long series only evoke response in a relatively small percentage of the motor neurones which ultimately take part if the stimuli are continued long enough. Gradually more and more fresh motor neurones are brought into action as the reflex develops. This process the authors designate "recruitment." Cooper and Adrian (8) have recently reported observations on the electric response of muscles in the same two reflexes, supporting the conclusions of Liddell and Sherrington. The fact already mentioned, that the crossed extension reflex is often not obtainable in response to a single stimulus, may be regarded as another aspect of the extreme sluggishness of this reflex in getting started. It is confirmed by the work of Liddell and Sherrington, in which they show that even when a response does occur to a single stimulus the resulting contraction bears a much smaller ratio to that evoked by continuous stimulation than is the case with either motor nerve stimulation or the flexion reflex.

Buytendyk (9) has reported a study of the electromyogram of mammalian extensor muscles in decerebrate rigidity and in the crossed extension reflex. He did not, however, examine this reflex as evoked by a single shock, nor did he make records from the motor nerve. We have devoted our attention chiefly to these two matters, only repeating Buytendyk's observations for purposes of comparison with our other records.

Method. The essential features of the method have been described in the previous papers of this series (1), (2), (3). Some of the experiments were performed with the same string galvanometer which was used in the first two papers of the series. In the more recent experiments we have used the Hindle galvanometer, as in the later group of experiments in the third paper of the series (3). In a majority of experiments we have connected the unaided galvanometer directly with the physiological preparation; in these we have used a slack string. In others we have employed the method of electron-tube amplification already described (10) and applied in some of the experiments described in the third paper.

In some experiments we stimulated with single induced shocks, breaking the primary current with a copper-mercury key (1, p. 132) operated by hand. Sometimes we repeated the stimuli by hand with frequencies up to 8 or 10 per second (counting make shocks), and in some experiments we applied stimuli of higher frequencies by interrupting the primary circuit with a rotary interrupter (11). In some experiments we have led off from the motor nerve damaged between the leads to render the responses monophasic. In others we have led off from the innervated muscle. In some of these experiments we have recorded simultaneously the action currents and the mechanical contraction of the muscles involved. For this purpose we generally worked with the gastrocnemius muscle, but occasionally have attempted to record responses of the knee extensors. For mechanical recording we have not attempted to use an isometric lever, for we were not concerned with any such refined analysis of the contractile process as Sherrington has made in his recent researches. We desired merely a mechanical indicator to show approximately the amount of contraction occurring under the various conditions of our experiment. For this purpose we arranged a light aluminum bell-crank lever to write on a smoked drum, its upward excursion being partly restrained by the tension of a rubber band. In two experiments we recorded mechanical contraction by means of a lever which threw a shadow on the photographic film beside that of the string in the galvanometer. In some experiments the time of stimulation was registered on the photographic film by means of the same signal magnet employed in the experiments described in the first paper of this series. In some of the more recent experiments a small string galvanometer, consisting of a permanent magnet with a light copper wire stretched through the field in the path of the beam of light, was

introduced in the primary circuit of the stimulating coil in place of the original signal magnet, in order to show the time of stimulation. In a few experiments the time of stimulation was not recorded.

As in all previous experiments recorded in this series of papers, the animals (cats in every case) were decerebrated under deep ether anesthesia. After decerebration was complete the ether was withdrawn. The sciatic nerve of one leg (usually the left) was exposed by a posterior incision, ligated in the popliteal space and severed distal to the ligature; stimulating electrodes shielded with glass (Sherrington shields) were applied to the central cut end for afferent stimulation, with the cathode on the break shock nearest the center. The operative procedure preparatory to recording extensor activity in the opposite hind leg varied according to the tissue to be used. The gastrocnemius muscle and its motor nerve (popliteal) were usually selected in preference to the knee extensor muscle or its motor nerve, in view of the greater accessibility of both muscle and nerve, and especially the greater length of the popliteal nerve available for recording. When we wished to record the action current of the motor nerve (popliteal) we severed this close to its entrance to the gastrocnemius muscle, damaged the fibers by crushing near the point of severance, and, as in the case of the flexion reflex, led the nerve into a moist chamber in which contact was made with two non-polarizable electrodes, the damaged point lying between them in order to render the responses monophasic.

In view of the great diminution of apparent electric response which results from recording diphasically, even in the case of the flexion reflex, in which the impulses are nearly synchronous (1, fig. 8), we made no attempt to record diphasic action currents in the crossed extension reflex, for the dispersion and overlapping of impulses would presumably be such as to cause an almost complete disappearance of any electric response.

The moist chamber was clamped rigidly to a separate stand in such a way that there should be no jarring of the chamber, such as might cause shift of electrode contact. When we sought to lead off the action currents of the gastrocnemius muscle we avoided, as far as possible, injuring the tissues by dissection, merely opening two small windows through the skin over the gastrocnemius muscle and attaching the wicks of two agar electrodes (previously described) to the muscle by sutures passed through the deep fascia over the muscle.

Observations: *Response in motor nerve.* As regards the mechanical response, we found great variation between individual animals in the readiness with which the crossed-extension reflex could be evoked, especially in the case of single induction shocks. Often a preparation would at first show the reflex in response to single shocks, but later would cease to do so, the reflex only appearing on application of a more or less prolonged series of stimuli. Out of 22 preparations studied, about 18 showed a

definite crossed-extension reflex in response to a single shock at any time. Of these only 12 continued to do so throughout the experiment. In the remaining preparations it became impossible during the course of the experiment to elicit the reflex except by repeated stimulation, usually about the time everything was in readiness to begin making records. When the reflex was evoked by a single shock it was apt to be of comparatively brief duration, lacking the conspicuously long after-discharge which characterizes it when evoked by repeated stimuli.

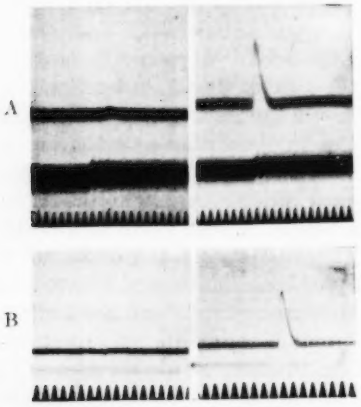


Fig. 1. String galvanometer records showing (1) monophasic response of extensor motor nerve to single maximal induction shocks applied to the afferent nerve of the opposite side and for comparison (2) records of monophasic response of the same motor nerve to direct maximal stimulation.

Time here, and wherever recorded in subsequent figures, shown by shadow of tuning fork; 1 double vibration = 0.01 second.

A, March 2, 1916. Cambridge galvanometer; 5,000-ohm, platinum string, diameter 3μ, tension 215 m. per amp. (see Forbes and Ray (43)). Signal magnet in stimulating circuit shown in middle line in which rise indicates break of current. Magnification 580.

B, November 15, 1923. Hindle galvanometer; 16,800-ohm, gilded quartz string, diameter 1.25μ, tension 204 m. per amp., 0.2 microfarad condenser damping in string circuit. Magnification 490.

The most striking feature in our electrical records made with the leads on the motor nerve was the extremely small size of the galvanometric excursions. In a majority of experiments we were unable to detect any excursion of the galvanometer at all, although the knee extensors revealed by contraction the presence of the reflex in response even to single stimuli. The largest excursions were much smaller than we have usually found under similar conditions in the case of the flexion reflex (1). Let us first consider the case of stimulation by single shocks. In figure 1 is a sample of the excursions obtained from the popliteal nerve in the experiment in which they were largest (A), together with a typical response in another experiment (B). In each case the response of the same nerve to direct stimulation, with the leads undisturbed, is shown for comparison beside the reflex response. Even the smaller of the two reflex responses shown in the figure is larger than we usually obtained.

In figure 1, A, the signal magnet enables us to measure the latency of the motor nerve response. In this case, and in several others like it

observed in the same experiment, the interval between stimulus and response was about 18σ. In other experiments in which the time of stimulation was recorded by a special galvanometer the latency was from 17 to 22σ; subtracting the conduction time in the peripheral nerve trunks, as was done in the case of the flexion reflex (1, p. 141), we may conclude that the reduced reflex time for the beginning of the crossed extension reflex is about 12 to 15σ.

The small size of these responses to single stimuli harmonizes with the observations of Liddell and Sherrington (7) as to the small percentage of motor neurones taking part in the beginning of the reflex response.

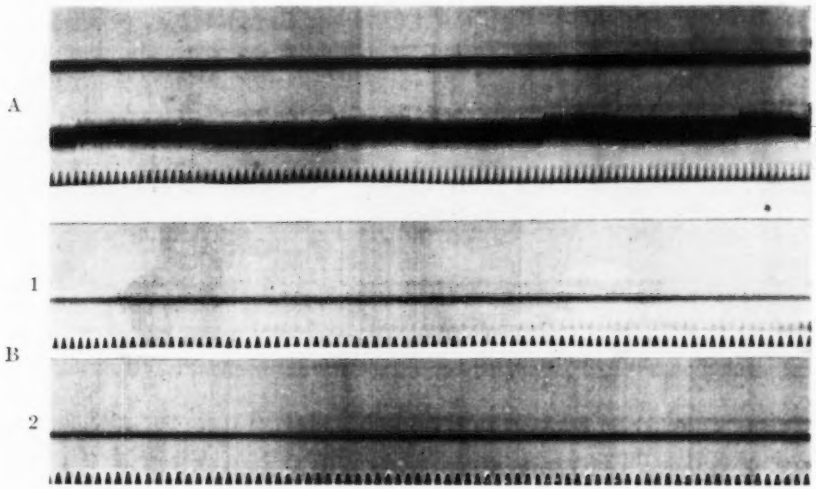


Fig. 2. Records made under the same conditions as in figure 1, showing responses of the motor nerve to repeated stimulation.

A, March 2, 1916.

B, November 15, 1923, (1), beginning of stimulation; (2), at height of reflex response (see horizontal white lines in figure 3).

With this consideration in mind we might suppose that concurrent with the process of "recruitment," i.e., when more motor neurones are brought into action, as revealed by increasing mechanical contraction in the knee extensors, we should find evidence of greater activity in the motor nerve revealed by the galvanometer. This is not the case. Figure 2 shows the galvanometric record during repeated stimulation in each of the two experiments which furnished the records in figure 1. In each case only at the very beginning of the reflex response does any galvanometric excursion appear. In both cases the presence of the reflex was revealed by knee extension. In the experiment of November 15 (fig. 2, B) knee exten-

sion was recorded graphically while the electrical record was being made. The mechanical tracing is shown in figure 3, with short horizontal lines indicating the time covered by the portions of the electrical record reproduced in figure 2, B.

It might be argued that the activity of the knee extensors, simultaneously observed, is not a valid criterion of discharge in the motor neurones innervating the gastrocnemius muscle, after the nerve has been damaged in order to render the action currents monophasic. It is well known that proprioceptive (afferent) impulses from the active muscle play an indispensable part in maintaining decerebrate rigidity (6). Moreover, the crossed extension reflex, with its sustained character, so resembles a mere increase in the state of decerebrate rigidity that we may well suppose the same proprioceptive impulses play a considerable part in the development of this reflex. Crushing the nerve of course stops this source of augmentation of central activity. May it not in this way prevent the reflex from developing altogether? The answer to this question is found in experiments performed with de-afferent preparations, that is, preparations in which the dorsal roots of the nerves supplying the extensor muscles have previously been sectioned. It is well established that in such a preparation, if time enough has been allowed for recovery from the operation, the crossed extension reflex can still be evoked (12), (13). The after-discharge is less than in the normal preparation, but it still is more marked than in the case of the flexion reflex (14, p. 130), (12). Responses not differing greatly from normal have been regularly evoked in the gastrocnemius muscle in a series of de-afferent preparations studied recently in this laboratory in connection with another problem, an account of which will presently appear. It is true that in this series it was somewhat difficult to evoke a crossed extension reflex when the dorsal roots had just been cut in the decerebrate preparation; the reflex was much less impaired when the preparation had been de-afferented aseptically under ether several days before. The inference might be that in the case of our experiments, in which the motor nerve was crushed and the afferent fibers were thereby interrupted immediately

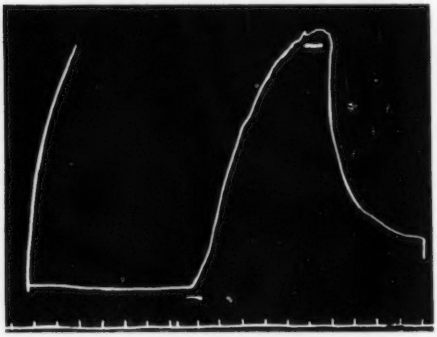


Fig. 3. Mechanical record of crossed extension reflex illustrating the stages of response to which the records in figure 2, B, correspond. Time was recorded by a Jacquet clock marking seconds. (November 15, 1923.)

before we examined the reflex, a condition existed similar to that which caused the failure of the reflex immediately after severance of the afferent roots. It might be that however the continuity of the afferent fibers from the extensor muscle is interrupted, the crossed reflex involving that muscle is greatly impaired and requires days for its recovery. But it seems to us more likely that the cause of the reflex's failure immediately after severance of the dorsal roots lay in the disturbance of the spinal cord, which inevitably occurs in such an operation, rather than in the mere interruption of the afferent path. This view is supported by the fact that in two "acute" experiments the reflex disappeared or was impaired in the knee extensors from which very few afferent fibers were severed, as well as in the gastrocnemius, which was completely de-afferented. It is also supported by the recent experiments of Adrian and Cooper with novocain (8). Therefore we may conclude that long as the interruption of the afferent path from the gastrocnemius was remote from the spinal cord in our experiments, and the cord was not exposed or disturbed, the motor

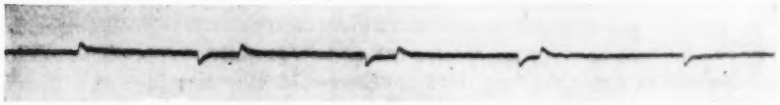


Fig. 4. Amplified galvanometer record from same experiment as in figure 2, B (November 15, 1923). The principal excursions are due to electrical artefact. Speed of film the same as in figure 2, B. String tension 85 m. per amp. Magnification 490.

neurones were probably not prevented from discharging their impulses in response to the appropriate stimulus in the opposite leg. We are probably justified in assuming that when activity in the extensor centers was revealed by contraction of the knee extensor muscles there was also corresponding activity in the motor neurones which innervate the gastrocnemius, even though of lessened amount because of the breaking of the afferent path.

If, then, we may assume that contraction in the knee extensors indicated the presence of motor nerve impulses in the popliteal nerve (innervating the gastrocnemius), we are confronted with the striking fact that as the discharge of impulses in this nerve increases in volume there is nothing in the galvanometer record to show for it. The records in figure 2 are fair samples. Although the activity in the center seems to have been increasing to a maximum as shown in one of these experiments in figure 3, no corresponding excursions appear in the galvanometer record, (fig. 2, B, 2).

We tried to see if the activity in the motor nerve during the crossed extension reflex could not be better shown with the aid of electron-tube amplification. Figure 4 shows the record thus obtained during a series of stimuli applied to the nerve with the key operated by hand. With the resistance in the grid circuit as great as that of the required length of nerve we were

unable to obtain a record with a perfectly smooth base line (cf. 10, fig; 26). The electrical artefact, due to the direct effect of the induction shocks upon the recording circuit, is the most conspicuous thing in this record; the make and break shocks are clearly revealed by the excursions of the string alternately in opposite directions. The absence of any other excursions appreciably greater than the fortuitous oscillations occurring in absence of any stimuli, corroborates the evidence in figure 2 of the paucity of measurable action currents in the motor nerve during the progress of this reflex.

What is the significance of this apparently complete lack of electric response in a motor nerve which we have every reason to suppose is actively engaged in conducting nerve impulses? It is true that only a small percentage of all the fibers in the popliteal nerve are motor fibers innervating the extensor muscles of the ankle,—probably about a quarter, in round numbers. We should naturally expect that such a small fraction of all the fibers in a nerve trunk, mixed with a majority of idle fibers, would register a much smaller response in the galvanometer than the entire nerve trunk would if stimulated as a whole, as has been done in the controls with which the reflex responses are compared in figure 1. It is worth while to determine approximately how much smaller the response of a small portion of the nerve trunk will be than that of the entire nerve. To this end we have performed a control experiment in which the entire nerve trunk was connected with the galvanometer, and first a branch supplying the gastrocnemius was stimulated, then the entire nerve. This involved reversing the direction of the impulses as compared with those in a reflex experiment, since the gastrocnemius branches could be separately excited only by applying stimulating electrodes to them distal to their point of branching, and with the leads on the entire nerve, this meant recording impulses conducted toward the center. But since it is well known that nerve fibers conduct in both directions, and no difference is known between the impulses passing in opposite directions in a given fiber, this reversal probably does not impair the control.

Sherrington has shown that from a third to a half of the fibers in a motor nerve are afferent (15); with direct stimulation we were exciting these as well as the motor fibers in the gastrocnemius branches. Therefore if we stimulated both branches supplying the gastrocnemius muscle our control would be a comparison of the response of about half the fibers in the popliteal nerve with that of the whole nerve, whereas the desired control involved stimulating only about a quarter of all the fibers. It is impossible to separate the motor fibers and excite them without exciting the afferent fibers at the same time, and this fact renders impossible an exact control; but by stimulating only one of the two approximately equal branches supplying the gastrocnemius muscle we obtained a fair approximation

to the proper number of fibers. We are probably not far wrong in assuming that all the fibers in one of these branches give somewhere near the same total response as the purely motor fibers in both branches would give if excited without the afferent fibers that are mixed with them. The result of this control is shown in figure 5. In A is shown the monophasic action current with leads applied to the whole popliteal nerve when only one of two branches to the gastrocnemius was excited with a maximal stimulus; in B is shown the response with the leads unchanged, but with a maximal stimulus applied to the whole nerve. It will be seen that although in A the stimulus was applied to barely a quarter of all the fibers in the nerve the galvanometric excursion is about one third as big as when the whole nerve was stimulated. Clearly the small number of fibers constituting the true motor innervation of the ankle extensors would not in itself account for the minuteness of the responses observed in the crossed extension reflex. The fact that three quarters of the fibers in the nerve trunk are idle would explain a reduction in the response comparable with that shown in figure 5, but it would not suffice to explain even the small size of the initial response in this reflex,—still less to explain the disappearance of even that small amount of electrical activity as the discharge of impulses becomes more general.

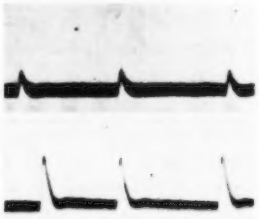


Fig. 5. Comparison of monophasic action currents in popliteal nerve with small fraction and with whole nerve maximally stimulated. Break shocks 204 Z units in each case (make shocks also maximal).

Hindle galvanometer; 17,000-ohm, gilded quartz string, 2.75μ diameter, tension 278 m. per amp.

A, Stimulus applied to about $\frac{1}{4}$ of nerve.

B, Stimulus applied to whole nerve.

Let us consider the duration of the individual action current in a nerve fiber. The best records of this are those of Gasser and Erlanger made with the Braun tube oscillograph (16). They show that at 25°C., which is approximately the temperature of the moist chamber into which our motor nerve was led, the peak of the electric response is reached in about 0.5σ after its first appearance, and in 1.5σ it is nearly over. The persistence of a slight electric disturbance as late as 4σ after the beginning is ascribed by them to the dispersion in time of the individual impulses in fibers conducting with different velocities.

We may safely conclude that at the temperature at which we worked, the time during which the action current in an individual fiber has an appreciable magnitude does not exceed 2σ. Almost all of the energy of the response manifests itself in less than 1σ. The popliteal nerve is made up of several thousand individual fibers. A response to maximal stimula-

tion of this nerve, such as is shown in figure 1 (A, 2 and B, 2), results from the occurrence of action currents simultaneously in all of the thousands of fibers in the nerve trunk. But if only very few of the fibers respond simultaneously very little current would be registered in the galvanometer. If the individual motor fibers are conducting impulses repeatedly, but at somewhat irregular intervals, and no two fibers are regularly keeping step with each other, all we should expect to find in the galvanometer record would be a very slight sustained deflection of the string, in consequence of the multitude of overlapping individual impulses. In other words, to obtain any such deflection of the string as is shown in the case of maximal stimulation we must have a large percentage of the individual fibers reenforçing each other by responding in unison. When the individual impulses get out of phase with each other the electrical response of the nerve as a whole becomes difficult to detect.

There is another possible factor in the apparent flattening of the electrical record as the discharge of motor impulses becomes increasingly general and continuous. Evidence has been reported from this laboratory (17) leading indirectly to the conclusion that in such sustained motor activity as voluntary contraction the nerve impulses in the individual motor fibers follow each other with so high a frequency that each occurs during the relative refractory period following its predecessor, and is therefore of subnormal magnitude. Further evidence in support of this view as regards the crossed extension reflex in the cat, has recently been found by an entirely different method, and will shortly be reported. If the majority of individual nerve impulses occur early in the relative refractory period following their predecessors, they must individually be small as compared with the full-sized response of a fiber stimulated after a period of rest. If, then, the total activity after the reflex discharge has become well established consists of these subnormal impulses, this fact may furnish an additional reason why the total disturbance recorded in the galvanometer should be very small.

We may conclude then that the absence of any well-defined excursion in the galvanometric record from the motor nerve during the height of activity such as occurs in the crossed extension reflex signifies that not enough individual fibers respond in unison at any one moment to give rise to a measurable deflection. Although a considerable number of neurones are engaged in conducting impulses and probably conducting them with a fairly high frequency, yet the individual impulses in the adjacent fibers are usually out of phase with each other, and thus do not show in the record.

In two experiments with rapidly repeated induction shocks we have found small excursions of the same frequency as the stimuli, gradually increasing in size during the first half second of stimulation. In one case

these excursions were at most only barely discernible; in the other they were so well defined that their time relations could be studied. Three examples of the responses in this experiment with different speeds of stimulation are shown in figure 6, together with the response of the same nerve to direct maximal stimulation. The signal magnet, showing the time of the individual stimuli, enables us to determine the latencies of the

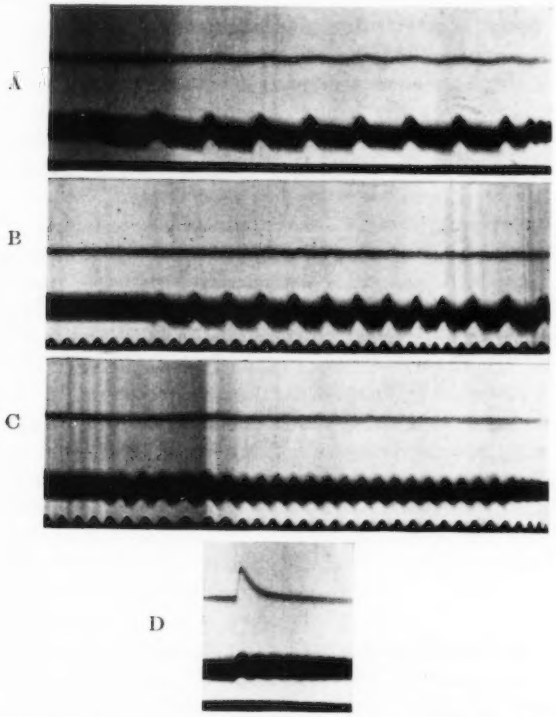


Fig. 6. Exceptional response of motor nerve in crossed extension reflex to repeated stimulation with frequencies shown by signal magnet (cf. fig. 1, A).

A, B and C, reflex responses; D, control record, maximal response of nerve to direct stimulation. Speed of film the same in all. Cambridge galvanometer; 28,000-ohm, silvered quartz string, 1.5μ in diameter, tension 145 m. per amp. Magnification 580.

separate responses appearing in the record; they appear to be about 7σ, measured from the break shocks, which are the stronger stimuli. This latency is strikingly less than that observed with single stimuli; indeed it is slightly less even than that previously reported in the case of the flexion reflex (1). Subtracting 4σ for conduction time in the nerve trunks, there would only be 3σ left for conduction time within the spinal cord. So brief a latency casts suspicion on the source of these galvanometric excursions.

In some experiments we have found regular oscillations which seemed only explicable as due to electrical artefact from the rotary interrupter. Control experiments in which this apparatus was connected directly with the galvanometer have revealed an electrical effect (probably static) which may, under certain conditions introduce a larger artefact than would result from the use of a simple key operated by hand. Part of the oscillation in the records shown in figure 6 may have been due to this artefact, but the fact that in every case the excursions increased gradually as the reflex developed, seems to exclude the electrical artefact as the sole cause. Another possibility is that as the reflex developed the muscle in its contraction vibrated in the rhythm of the interrupter, and the vibrations were transmitted to the nerve at the point of contact with the leads. A slight disturbance of electrode contact would suffice to cause small galvanometric excursions.

The question then is whether these excursions were due to a mechanical artefact (jarring of electrode contact) or to functional responses in the nerve. In figure 6, A, there are comparatively gradual undulations of the string suggestive of mechanical jarring, but superimposed upon these are sharper excursions, whose latency after the break shocks we have found to be about 7σ, and their direction is that which would result from action currents in the nerve. In figure 6, B, the only excursions are the abrupt ones in the proper direction for action currents, also following the break shocks by 7σ, although the speed of the interrupter was higher than in figure 6, A. These facts are strongly suggestive of action currents as the cause of the deflections. If we may so interpret them, the apparent decrease in latency from 18σ, in the case of a single stimulus, to 7σ, in the case of the successive stimuli of a rapid series, is a most striking and interesting phenomenon, indicating an actual decrease in conduction time across the spinal cord.

We cannot be certain that these excursions were not due to shift of electrode contact, although the moist chamber into which the nerve was led was so fixed that any such vibration must have been very slight. If they represent real functional responses in the nerve, the question arises why similar excursions were not found in all experiments. In that case we can only conclude that in this particular experiment, and, to some extent, in the other one mentioned above, enough neurones responded in unison after each stimulus to produce a visible excursion. We may suppose that in most of our experiments sustained activity in such a reflex signified a condition in which the motor neurones were being excited by impulses converging upon them through many branches of internuncial neurones, and were therefore discharging impulses repeatedly with so high a frequency that they were refractory to further stimulation most of the time. If this was the case, and if the individual motor neurones were responding

out of phase with each other, we should not expect to find galvanometric excursions correlated with the individual stimuli. The presence in this particular experiment (fig. 6) of excursions following the individual stimuli, suggests an unusual absence of after-discharge in the form of impulses converging upon the motor neurones from "delay paths" (18, p. 397), and consequently an unusual freedom of the motor neurones to respond to impulses reaching them by the most direct path from the afferent neurones. It is unfortunate that we have not more evidence to help us determine with certainty whether these excursions are in reality of physiological significance, but the rarity of this type of response in our experience renders it difficult to settle the question.

It is interesting in this connection to note that Cooper and Adrian (8), recording the electric response of a knee extensor muscle, found in most of their experiments "primary waves" corresponding in frequency with the stimuli up to 80 per second. This implies a definite grouping of motor nerve impulses such as would probably appear in the nerve record made under the conditions of our experiments (fig. 6). In some of their experiments this correspondence of frequency between response and stimulation did not appear. This matter will be discussed in connection with muscle responses in our experiments.

Responses of extensor muscles. Occasionally we have made records from the extensor muscles of the knee. These have usually been rather unsatisfactory. The probable reason is that the superficial muscle, to which the electrodes were usually attached, is the sartorius, and only a small portion of this participates in knee extension (19). We have obtained more satisfactory records by using the gastrocnemius muscle. Any measurable contraction in this muscle due to reflex innervation regularly reveals itself in a galvanometric record.

The gastrocnemius muscle is electrically a much more sensitive indicator of reflex activity than the motor nerve. There are two probable reasons for this: One is the greater duration of the individual action current in muscle, enabling the string to follow the electrical disturbance more nearly than it can in the case of the extremely brief action current of nerve. The other is the fact that each nerve fiber branches and innervates a number of muscle fibers (Lucas found the number to be about 20 in a certain amphibian muscle); thus the muscle serves as a sort of amplifier for the electrical effect. This is well illustrated in figures 11 and 12 of the first paper of this series (1) in which the response of the tibialis anticus muscle is compared with that of its motor nerve, both in the case of reflex and of motor nerve stimulation. Another striking example of this amplification by the muscle is shown in figure 7, in which the action currents of the gastrocnemius muscle, set up by rapid severance of its motor nerve with sharp scissors, are recorded with the aid of electron-tube amplification. Persistent

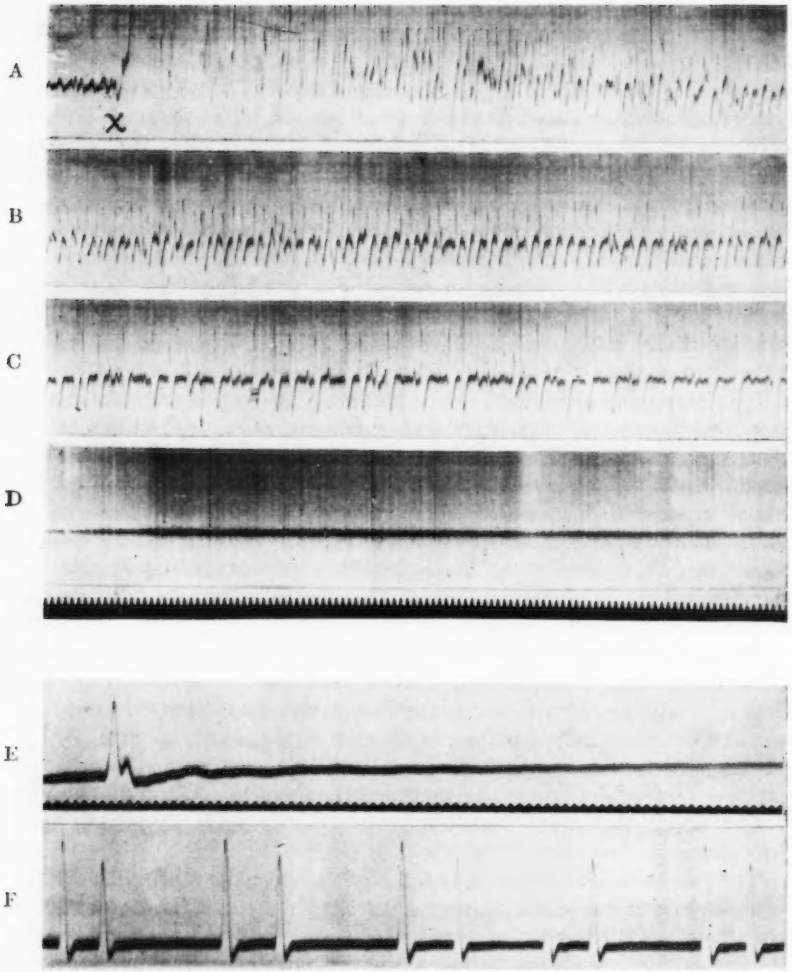


Fig. 7. Example of the greater sensitivity of muscle than nerve as an indicator of functional activity.

A, B, C and D, responses of gastrocnemius muscle to cutting of popliteal nerve. A, begins with muscle in decerebrate rigidity; *x* shows when nerve was cut. B begins 0.8 second after cutting. C, 1.9 seconds after cutting. D, about 45 seconds after cutting. Speed of film constant A-D.

Hindle galvanometer; 25,000-ohm, gilded quartz string, 1.5μ diameter, tension 51 m. per amp. Electron-tube amplification, with 0.1 microfarad condenser damping. Magnification 490. (April 21, 1921.)

E, monophasic response of popliteal nerve to cutting, and F, to direct electrical stimulation (break shocks maximal) immediately before cutting, for comparison.

Hindle galvanometer; 17,000-ohm, gilded quartz string, 2.75μ diameter, tension 122 m. per amp. Electron-tube amplification, with 0.2 microfarad condenser damping. Magnification 350. (May 9, 1924.)

responses are seen with gradually decreasing amplitude and frequency throughout the 5 seconds after severance, during which the film was exposed (A, B and C). The absence of any oscillations of the string due to artefacts, in the case of a low-resistance tissue such as muscle, is shown by the control (D) taken about 45 seconds after the nerve was cut, when evidently nerve impulses had ceased to be set up by the injury. In contrast to the record made from the muscle is a similar record made from a nerve upon rapid severance, also amplified with the electron-tube. This is reproduced in figure 7, E. It will be seen that in this case the string returned to its base line and ceased to show oscillations in less than a quarter of a second after the nerve was cut. Presumably nerve impulses were still arising from the injured point for a much longer time than this, as in the case in which their effect was revealed by muscular response, but the galvanometer fails to show them, probably because of their getting so completely out of phase with each other.

Before considering the response of the muscle in this reflex to single stimuli, let us first consider the electromyogram during the sustained contraction at the height of the reflex, for the condition here is similar to that of Buytendyk's observations (9) and to those of Cooper and Adrian (8), and we shall therefore be starting on familiar ground. In our experiments when a sustained reflex was evoked by repeated stimulation the resulting electromyogram from the gastrocnemius has resembled in a general way those of Buytendyk. But whereas the action current frequency appearing in his records is in the neighborhood of 70 per second, we have found higher frequencies more nearly resembling those which Adrian and Olmsted found in the ankle flexor muscle of the cat when reflexly excited by stimuli repeated more than 200 times a second (20), and which Cooper and Adrian have found in both reflexes with like frequency of stimulation (8).

In discussing our motor nerve records we have already mentioned the fact that Cooper and Adrian, in recording the electromyogram of the vasto-crureus muscle in the crossed extension reflex have in most of their experiments found "primary waves," i.e., electric responses corresponding in frequency with the stimuli, when these were applied not more than about 80 per second; in a few experiments they have obtained with all frequencies of stimulation only the irregular rhythm characteristic of the voluntary electromyogram, similar to that appearing in Buytendyk's records and in all of their own records in which the stimulus frequency was high (e.g., over 100 per second).

In examining our records from extensor muscles, we find only one experiment in which this correlation of frequency between responses and stimuli appears. Figure 8 shows an example of the responses obtained from the knee extensor in this experiment, with 38 stimuli per second, in which major electric responses regularly followed the rhythm of the stimuli.

Other observations in this experiment lead us to suspect that the electrical artefact already mentioned as peculiar to the rotary interrupter may have contributed to the regular excursions here recorded, but their size and expecially the inequality of their size make it certain that they must be chiefly due to action currents in the muscle. In the rest of our experiments with all frequencies of stimuli employed, including 4 to 10 a second by hand and 30 to 240 a second with the rotary interrupter, we find only the irregular type of rhythm which is found in voluntary contraction, in Buytendyk's observations, in those of Cooper and Adrian with stimulus-frequencies above 100 per second, and in the case of three of their experiments with all frequencies of stimulation.

It is a curious fact that we should have found in only one instance a phenomenon which, under apparently similar conditions, was found in the majority of theirs. The significance of this difference is not apparent. It is noteworthy that individual animals have often been found to show strik-

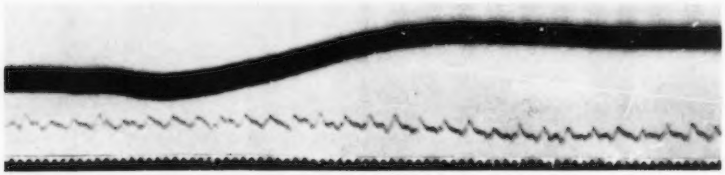


Fig. 8. Electromyogram in which a correlation between frequency of response and stimuli was found. Rotary interrupter making 38 contacts per second. Leads on knee extensors and stimulating electrodes on the opposite sciatic. Extension of knee recorded on the film, by rise of upper line.

Cambridge galvanometer; 12,000-ohm, gilded quartz string, 1.5μ diameter, tension 160 m. per amp. Electron-tube amplification. Magnification 303. (June 7, 1920.)

ing differences in the characters of their reflex responses to various stimuli. The causes of these are not understood. Some are probably due to inherent variations in the animals. Others are probably due to uncontrolled divergences in experimental procedure, resulting perhaps in differences in blood pressure or temperature or other aspects of physiological condition. It may well be that some unrecorded difference in the methods of preparation employed by two groups of investigators caused in some unknown way a fairly consistent difference in the type of response obtained.

Returning to the divergence in frequency between Buytendyk's records and ours, before concluding that it signifies any real difference in physiological activity, let us consider certain factors which probably determine the frequency of oscillations that can be detected in a string galvanometer record. A typical electromyogram such as appears in his records or ours, presents a very irregular rhythm; occasionally the oscillations are for a

short time fairly large and regular, but in other portions of the record they vary greatly in size and duration. This suggests that at times the muscle fibers respond more nearly in phase with each other than at other times. If we attempt to count the oscillations their number will depend on how small a group of approximately synchronous individual action currents the recording apparatus is capable of registering. This must depend on the lightness of the string, the magnification, the sharpness of focus and the speed of the photographic film or plate, the latter having an optimum range of speeds for a given type of record. Of these factors usually the most important is the lightness of the string, for a feeble force of very brief duration will not produce perceptible excursion of a heavy string, even with the best optical system to be had. This point is illustrated by a comparison of our records made with four different strings in the same galvanometer. In each case we used the gastrocnemius muscle, and the experimental conditions were essentially the same except for the mass of the string. In two cases the afferent nerve was stimulated by means of the rotary interrupter, in the other two cases it was stimulated by manual interrup-

TABLE 1

DATE	DIAMETER OF STRING IN μ	RELATIVE MASS	DOMINANT RHYTHM	TOTAL FREQUENCY
January 24, 1924	2.75	4.85	85–140	140
October 27, 1922	2.0	2.56	115	145
January 24, 1921	1.5	1.44	100–155	202
December 18, 1923	1.25	1.0	140–175	245

tion of the primary current at a rate of four or five a second (with make as well as break shocks effective, this means 8 or 10 stimuli per second). It seemed to make little difference in the type of response which method of stimulation was used. In figure 9 are shown the electromyograms from these four experiments; in the case of the lightest string we have enlarged the record from the original film in order to render the finer oscillations more clearly visible. In table 1 are shown the oscillation frequencies found in these records, together with the diameters of the strings and their approximate relative masses, using that of the lightest as a unit. Under "dominant rhythm" are given the frequencies of the larger and more regular oscillations, and in this there is often fairly wide variation between different parts of the same record. Under "total frequency" are included all the oscillations we could count. The dominant rhythm, depending probably on a true rhythm of large groups of fibers when responding in phase with each other, varies comparatively little with the mass of the string; all of these strings were light enough to record it. This rhythm may differ, though probably not greatly, between different physiological preparations. The total frequency, on the other hand, varies definitely

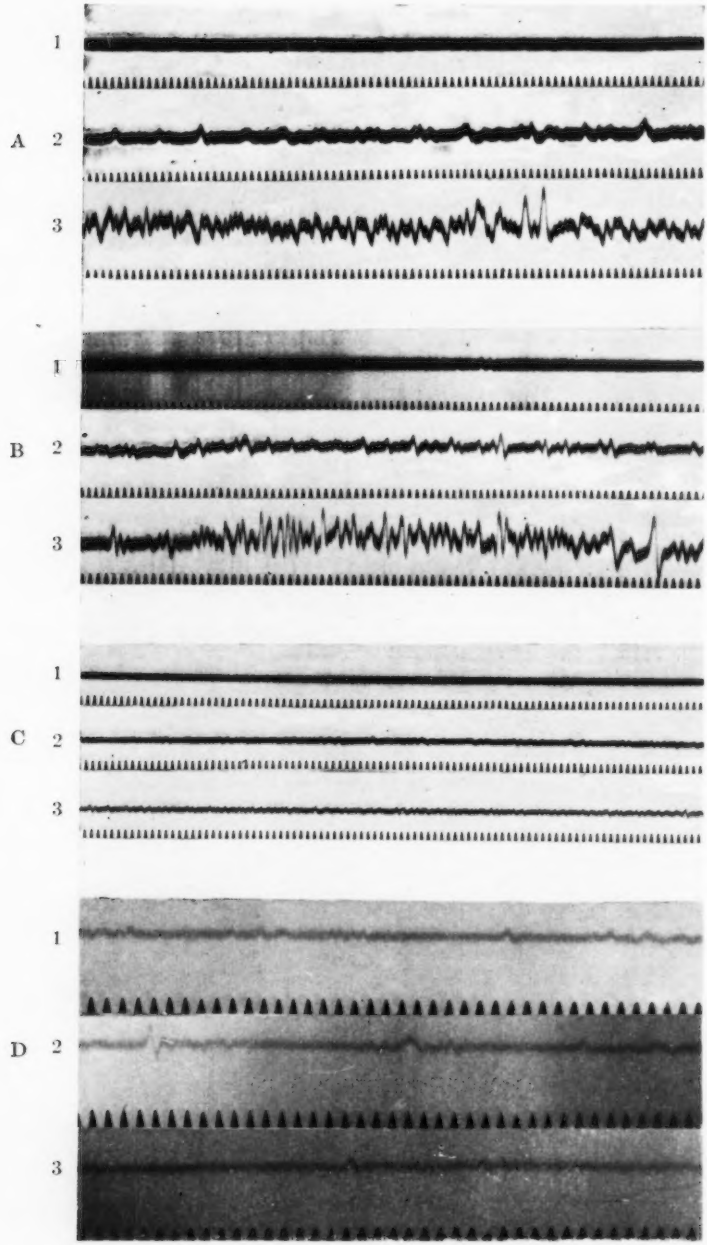


Fig. 9

and widely with the mass of the string, a fact which is easily explained as intimated above, by the irregularity with which groups of fibers get in and out of phase with each other, the number of oscillations discernible depending on how small a group of individual action currents the string can reveal with the definition rendered available by the optical system.

It is noteworthy that in all of our experiments the frequencies observed were considerably higher than that reported by Buytendyk. It should also be noted that he used a platinum string whose mass was probably about fifteen times as great as our lightest and three times as great as our heaviest. The magnetic field of the Edelmann instrument which he used was also probably less than that of the Hindle instrument used in our experiments, and the sensitivity was correspondingly less on that account. These facts probably in large measure explain the difference in frequency in his records and in ours. There may also have been a slight difference between the physiological behavior of his preparations and ours; but this difference was probably insignificant compared with that of inertia in the strings.

In the case of figure 9, C and D, the mechanical records taken simultaneously are reproduced in figure 10, and the parts of the record from which the electromyogram has been reproduced are indicated by horizontal white lines. One of these records (C) is taken from an experiment intended primarily to demonstrate a somewhat different point from that which is the subject of the present paper. It was one in a series of which a preliminary report has already appeared (21), and of which a more complete report will be published shortly. In these experiments we not merely stimulated the afferent nerve in the opposite leg, but, by means of a separate coil, we stimulated the motor nerve of the gastrocnemius muscle before, during and after reflex excitation. The responses to these inter-current stimuli are seen in the sharp spikes representing the maximal twitch evoked by a single stimulus when the muscle was not being reflexly excited, and later

Fig. 9. Electromyograms of decerebrate rigidity and crossed extension reflex showing variations in size and frequency of action currents when recording with different strings. (1) Records in absence of stimulation; (2) at beginning of reflex response; (3) at height of contraction. In each experiment leads were on gastrocnemius muscle, and the Hindle galvanometer with gilded quartz string was used.

A, January 24, 1924. 17,000-ohm string, 2.75μ diameter, tension 174 m. per amp. Magnification 490.

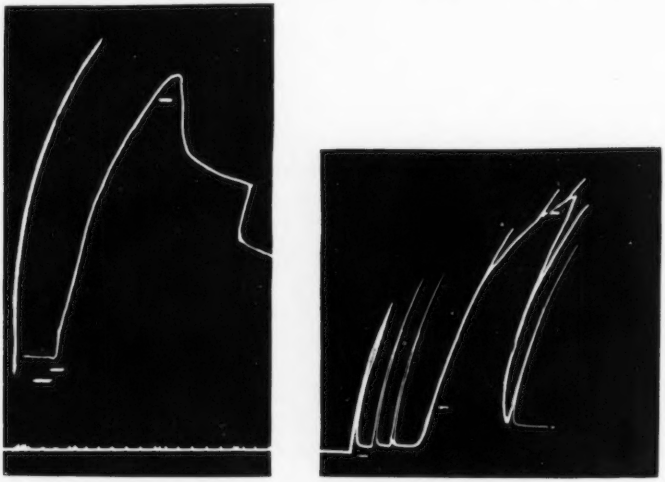
B, October 27, 1922. 4,800-ohm string, 2μ diameter, tension 60 m. per amp. Rotary interrupter in stimulating circuit. Magnification 490.

C, January 24, 1921. 19,500-ohm string, 1.5μ diameter, tension 41 m. per amp. Rotary interrupter in stimulating circuit. 0.1 microfarad condenser damping. Magnification 490.

D, December 18, 1923. 16,800-ohm string, 1.25μ diameter, tension 170 m. per amp. Magnification 858.

such response as was evoked by the same stimulus, applied directly to the motor nerve during the course of the reflex. This feature of the records has a bearing on the present research which will be discussed presently.

Buytendyk found that the electromyogram during the height of the reflex usually differed from that obtained during the preëxisting decerebrate rigidity only in the amplitude of the electrical oscillations, their frequency remaining about the same. In figure 9 we have shown in the top record from each experiment the electromyogram obtained in absence



A B

Fig. 10. Mechanical records of crossed extension reflex corresponding to records in figure 9, C and D. Short, horizontal, white lines show portions of the experiments given in figure 9.

A, December 18, 1923. Jacquet clock marking seconds.

B. January 24, 1921. Responses to motor nerve as well as to reflex stimulation are shown (see text).

of stimulation of the crossed afferent nerve. This represents such activity as was present in the extensor muscle in consequence of the decerebrate condition. In three of these action currents may be seen; in the other one no galvanometric excursions can be detected. In those showing action currents their frequency appears conspicuously lower than at the height of the crossed extension reflex. This result at first sight seems at variance with that of Buytendyk, but the discrepancy is probably due to the fact that our preparations did not manifest decerebrate rigidity in such high degree as did his. With feeble rigidity action currents may have occurred in groups of fibers too small to produce a perceptible excursion of the string. Indeed, the preparation in our series that showed no excursions (January 24, 1921) was definitely found to have a slight degree of

decerebrate "tonus," by comparing the angles of the ankle joint under carefully controlled mechanical conditions before and after section of the popliteal nerve. This means that action currents would have been found had the apparatus been sensitive enough to detect them.

In one experiment we repeated Buytendyk's procedure of stretching the gastrocnemius muscle by tension on the tendon during moderate decerebrate rigidity and recording the action currents. In two respects we varied Buytendyk's procedure:—we applied the tension gently at first, increasing it gradually to a measured maximum, and we amplified the electric responses with the electron-tube arrangement. It was the same experiment as that which furnished the records in figure 7; indeed it was taken

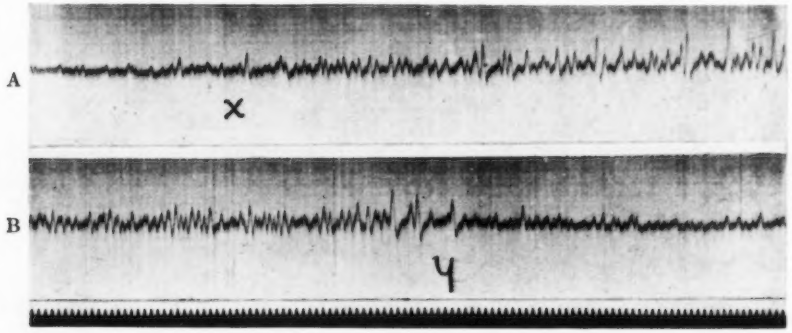


Fig. 11. Amplified electromyograms showing effect of stretching gastrocnemius muscle during decerebrate rigidity. In A, stretching begins at x and gradually increases. B begins with applied tension still increasing to maximum (2200 grams); tension ceases at y.

Hindle galvanometer; 25,000-ohm, gilded quartz string, 1.5μ diameter, tension 51 m. per amp. Electron-tube amplification with 0.1 microfarad condenser damping. Magnification 490. (April 21, 1921.)

immediately before these, and neither the electrodes nor the recording apparatus were disturbed between the two observations. The results are given in figure 11, showing the response at the beginning of the stretching (A), and at its conclusion (B). The maximum tension on the tendon, just before the tension ceased, was approximately 2200 grams, applied by pulling with a spring balance tied to a point on the foot 3.7 times as far from the ankle joint as the attachment of the Achilles tendon, till it registered 600 grams. The electron-tube amplification enables us to detect smaller action currents than we can in any of the unamplified records.

Superficially the frequency of oscillations appears much greater during the application of tension than before or after, but if we count every visible excursion in these records we find their frequency throughout lies between

150 and 175 per second. This experiment seems, then, to confirm the results of Buytendyk, as regards the approximately constant frequency with and without tension.

The second record of each experiment shown in figure 9 is the electromyogram during the beginning of the reflex in response to a rapid series of stimuli, delivered either by hand or with higher frequency by the rotary interrupter. The stages of mechanical contraction in two of the experiments are shown by the horizontal white lines in figure 10.

It will be seen that the reflex response in the muscle begins with isolated groups of electrical oscillations at somewhat irregular intervals with periods of complete or almost complete inactivity between them. The oscillations within each group have about the same frequency that prevails when the reflex response has reached its height. The groups of oscillations become gradually more frequent until, at the height of contraction, the record

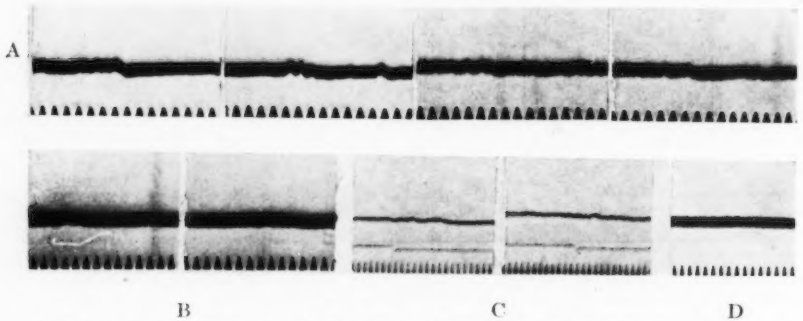


Fig. 12. Electromyograms showing crossed extension reflex in response to single stimuli.

A, March 8, 1922. Hindle galvanometer; 17,000-ohm, gilded quartz string, 2.75μ diameter, tension 174 m. per amp. Magnification 490. Leads on gastrocnemius muscle.

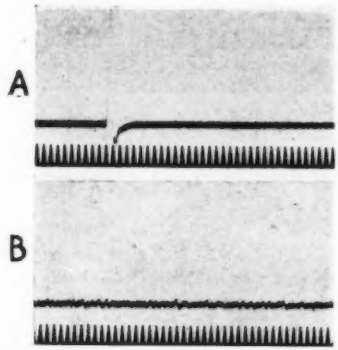
B, March 30, 1923. Hindle galvanometer; 17,000-ohm gilded quartz string, 2.75μ diameter, tension 347 m. per amp. Magnification 490. Leads on vasto-crureus muscle.

C, June 10, 1919. Cambridge galvanometer; 20,000-ohm, gilded quartz string, 1.5μ diameter, tension 330 m. per amp. Magnification 303. Leads on vasto-crureus muscle.

D, January 24, 1924. Hindle galvanometer; 17,000-ohm, gilded quartz string, 2.75μ diameter, tension 174 m. per amp. Magnification 490. Leads on gastrocnemius muscle.

shows the typical form of the electromyogram in any sustained contraction, the string being almost constantly in oscillation with the irregular rhythm we have already discussed.

When it comes to recording the reflex response of the extensor muscle to a single stimulus it is more difficult to obtain satisfactory results. If



In the paper by FORBES AND CATTELL in the September number of this volume, figure 13 on p. 162 was defective, the significant part of the figure having been cut off in reproduction. The accompanying corrected figure should be pasted over the original.

there is enough decerebrate rigidity to make action currents appear in the record then it is difficult to distinguish those evoked by the individual stimulus from those already present in consequence of decerebrate rigidity. If the decerebrate rigidity is too feeble to give rise to confusing action currents there is usually little or no crossed extension reflex in response to a single stimulus.

We have, however, obtained a few good records in which a definite response was evoked in the gastrocnemius muscle by a single shock (applied to the opposite sciatic nerve). Examples of this are shown in figure 12, together with two records from the knee extensors. They regularly reveal a series of action currents following each other with the frequency characteristic of electromyograms in the case of sustained activity, as in the reflex evoked by a continued series of stimuli. Apparent exceptions to this are found in the knee extensor records (C) of figure 12, in which there appears to be but a single excursion. But close examination of the film reveals a series of very small excursions following the major one. It is, of course, conceivable that each oscillation in the record represents the action current of a separate group of fibers, that none of these groups responded twice, and that the dispersion in time represents differences in reflex conduction time in the various individual arcs. But since the rhythm is so characteristic of that occurring in more prolonged activity it seems far more reasonable to ascribe it to repetitive discharge of impulses in the individual neurones. This view is supported by Sherrington's evidence that even in the much briefer flexion reflex there is commonly a repetitive discharge of motor neurones in response to a single stimulus (4), and by the evidence of Cooper and Adrian in connection with their "secondary waves" (8). It is also in harmony with the view that the branching arrangement of the central nervous connections is such as to preclude the conception of isolated individual reflex arcs (cf. 18, p. 377), a view supported by the evidence of Liddell and Sherrington (13) that as this reflex progresses increasing numbers of motor neurones become involved in the response to successive afferent stimuli.

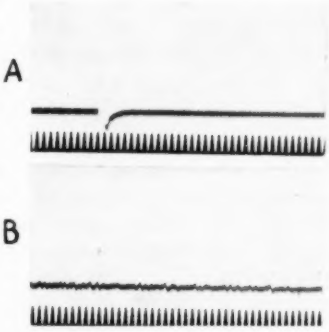


Fig. 13. Comparison of response of gastrocnemius muscle to maximal stimuli applied to motor nerve with response of same muscle during height of crossed extension reflex. (January 24, 1921.) The electrical record (A) corresponds with the second sharp excursion of the writing point in figure 10, B. The reflex electrical record (B) corresponds with the peak of the mechanical record in figure 10, B. For data as to string, see figure 9, C.

One of the most significant things which should be observed in the above records of muscle responses is the small size of the recorded action currents during the height of mechanical contraction of reflex origin as compared with that obtained with a single maximal twitch due to stimulation of the motor nerve. A record of this maximal action current on motor nerve stimulation is shown in figure 13, together with a reflex response recorded immediately afterwards. The high velocity of the string renders the photographic record of the former response somewhat indistinct, but a careful examination of the figure will reveal the peak of the electrical response 17 mm. from the base line. In contrast with this relatively large excursion it is interesting to note that the maximum excursion found during the height of reflex activity amounted to barely half a millimeter, or less than a thirtieth of the maximal response, and yet when we inspect the simultaneous mechanical records (reproduced in figure 10, B) we note that at the height of reflex response the contraction is considerably greater than the maximal twitch in response to a single stimulus applied to the motor nerve. Presumably the maximal stimulus of the motor nerve excited every fiber in the muscle, causing all to respond in unison. And yet the *mechanical* effect is small compared with that which occurs during the sustained reflex, when the *electric* responses appear exceedingly minute compared with that marking the perfectly synchronous appearance of the action currents of all fibers together. The effect of perfect synchronism seems to be a great increase in the electric response as revealed in the recording instrument, without any similar increase in mechanical efficiency.

Even supposing the individual muscle responses in the reflex occur with such frequency that each is in the relative refractory phase following the preceding response, and is therefore subnormal, this fact could hardly account for the enormous discrepancies between the size of electric response shown when all the fibers develop their action currents simultaneously, and that found in the reflex records. We must conclude that during reflex activity only a small percentage of all the fibers in the muscle responds at any given instant. Probably individual fiber groups respond in alternation or rotation, and the resulting mechanical effect appears to be highly efficient, judging from the contraction as actually recorded on the smoked drum. The small size of the electrical response in muscle during sustained reflex activity serves to reinforce the conclusion already arrived at from a study of the electric response, or lack of response, in nerve under similar conditions. In spite of the favorable conditions already mentioned for detecting responses in muscle as compared with nerve, the excursions are so small that we are led by them to the same conclusion suggested by the nerve record, that at any given moment only relatively few fibers are responding in unison.

The above facts present an interesting contrast to the observation of Piper on the human electromyogram (22). He found that in strong voluntary contraction of the forearm flexors the individual waves in the electromyogram were about the same, both in time relations and in amplitude, as those obtained upon maximal stimulation of the motor nerve through the skin with a single induction shock. It is true that in some examples of the crossed extension reflex (e.g., fig. 9, A and B, and Buytendyk (9) fig. 4) the action currents appear much larger than in the case illustrated in fig. 13 but in our experience they are always small compared with the maximal response to motor nerve stimulation. This fact suggests a high degree of mechanical efficiency in the execution of the crossed extension reflex. The economy of energy in the maintenance of decerebrate rigidity has been shown to be extraordinary (23), (24), and this bit of evidence of economy in the crossed extension reflex is interesting in connection with the view already suggested by the electrical evidence of Buytendyk, that this reflex differs from decerebrate rigidity in intensity rather than in kind.

Some investigators, e. g., Salamonson (25), have held the view that there is a "tonus" in skeletal muscle, distinct from ordinary contraction, and devoid of action currents. It might be suggested that when the crossed extension reflex exhibits the remarkable efficiency just mentioned (strong sustained contraction with surprisingly small action currents), it is because the "tonic" activity is brought into play (cf. 26). It seems to us that the theory of such a dual function in skeletal muscle is without adequate foundation (cf. 18, p. 403). Moreover, our electron-tube records lead us to believe that even in the feeblest degree of decerebrate rigidity action currents can be shown if the recording apparatus is sufficiently sensitive, a fact which tends to make all forms of contraction in skeletal muscle appear dependent on the type of response which follows ordinary stimulation, and which is inevitably characterized by action current.

Discussion. The most striking fact brought out by our experiments is the extreme difficulty in recording with a galvanometer what is going on in a motor nerve during the reflex activity which manifests itself as sustained contraction of a muscle. This leads us to a consideration of the observations of Athanasiu (27) in which he claims to have recorded rhythmic action currents from motor nerves engaged in similar activity, not only when the leads were applied directly to the nerve but when they were merely applied to the contracting muscle.

Before proceeding to consider the bearing of our observations on the interpretation of those of Athanasiu we feel that we should mention certain arguments of this author which have a general theoretical bearing on the problems involved in the study of reflexes in general. In describing and endeavoring to interpret our results we have tacitly assumed the

all-or-nothing law to be established for the functional responses of both nerve and skeletal muscle. First we should state what we mean by the all-or-nothing law. It has been subject to widespread misinterpretation by some physiologists who have assumed it to state more than it does. This law, as it has emerged from the work of Gotch (28), Lucas (29), Pratt (30), Adrian (31) and others, simply means that the propagated disturbance evoked by a single stimulus in a single functional unit of nerve or muscle is always as large as that functional unit is capable of producing at the moment when the response is evoked, no matter how strong the stimulus may be. In other words, the size of the propagated disturbance is independent of the strength of the stimulus, provided this is adequate. The law does not state that no feature of the response can be modified by the conditions under which the tissue finds itself when the stimulus is applied. Hill (32) and Fenn (33) have both pointed out that the energy developed by muscle in response to a given stimulus may be altered by conditions imposed on the muscle both before and after the moment of stimulation. But these facts do not alter the fundamental law that the size of the propagated disturbance is independent of the strength of stimulus.

Confusion has resulted from confounding the mechanical shortening of a muscle with the propagated disturbance. It is true the mechanical contraction is often a convenient measure of the propagated disturbance, and indeed was used as such by Lucas and Pratt in the experiments in which they adduced the strongest evidence in support of the all-or-nothing law for muscle fibers. The propagated disturbance is a phenomenon which appears to be essentially the same in nerve and muscle. In each case its progress along the fiber is marked by the electric response or action current. Mechanical contraction is a phenomenon peculiar to muscle in which it follows as a consequence of the progagated disturbance. The distinction between the propagated disturbance and the mechanical contraction is clearly shown in two ways,—one by a comparison of durations in the case of a single response, the other by a study of tetanic or sustained contraction. In the frog's sartorius at 20°C. the action current which marks the presence of the propagated disturbance has a duration at a given point of about 6σ. The mechanical contraction in the simple twitch resulting from a single propagated disturbance has a duration of about 80σ, or twelve times as long as the propagated disturbance. When a muscle is tetanized the mechanical contraction is perfectly fused into a single state of shortening, whereas a string galvanometer will reveal a series of perfectly distinct propagated disturbances, each one a unit of functional response in itself.

Athanasiu maintained that the all-or-nothing law is not valid in the case of muscle fibers, on the ground that a single fiber excited by the pore

electrode, as in the experiment of Pratt, clearly cannot attain its maximum possible shortening on account of the resistance to its motion which must be imposed by the idle fibers about it. Anyone acquainted with the work of Lucas and of Pratt is well aware that these authors never maintained that a single fiber contracting by itself could shorten as much as it can if neighboring fibers are shortening at the same time and thereby aiding it. Anyone who has read and understood their papers will see at once that Athanasiu's contention has nothing whatever to do with the all-or-nothing law, which is really concerned with the size of the propagated disturbance. In their experiments mechanical shortening was the immediate criterion but only simple twitches were considered; the authors both recognized that a rapid sequence of stimuli could evoke *summated contraction* greatly in excess of the maximal simple twitch. It may be properly said that contraction served merely as an indicator for the magnitude of the underlying disturbance, and inasmuch as their experiments were properly controlled by the avoidance of temporal summation, contraction in these instances constituted a perfectly valid indicator.

Adrian has since reinforced Pratt's experiment and confirmed his conclusions by stimulating a muscle fiber with the pore electrode and recording the electrical instead of the mechanical response (34). Lapicque (35) has already answered the argument of Athanasiu so clearly that further discussion of the point might seem superfluous. Yet the question is so important that it seems worth summarizing the discussion for the sake of those who may possibly be under some misapprehension in the matter.

Athanasiu also contends that all the responses in the individual fibers of a muscle executing a normal contraction must occur in unison if the muscle is to contract efficiently. He likens a muscle in which the fibers respond in alternation or rotation, as Lapicque has suggested they do, to a group of men pulling on a rope in rotation instead of in unison, as an argument for the necessity of response in unison. In this argument the same misconception already mentioned is made, namely, the confounding of the propagated disturbance, or the action current which marks its presence, with mechanical contraction. This mechanical contraction is perfectly sustained even if the successive propagated disturbances in the fibers are separated by as much as 10σ at mammalian body temperature; and since separate action currents in a muscle at this temperature can follow each other by as little as 3σ, this argument falls to the ground. We must depend on experimental evidence and arguments of quite a different sort to determine whether the individual action currents in a muscle engaged in sustained reflex action occur in unison or in alternation and rotation.

Let us now consider the bearing of our observations on the interpretation of those of Athanasiu. This author finds in his electromyograms large oscillations of frequencies from 70 to 150 per second and small oscillations

of from 300 to 500 per second. He says the large ones are muscle action currents and the small ones nerve action currents. The weakness of his interpretation stands out clearly in its application to his figure 2, plate 1 (27). In this record he notes that the beginning of contraction is heralded by a series of small oscillations which he says are due to activity in the nerve, preceding as they do, appropriately enough, the larger oscillations which mark the beginning of activity in the muscle, but a careful examination of this record shows that there are about 7 of the smaller oscillations apparent in the record before the first of the larger ones. Now it is well established that when a motor nerve is stimulated an impulse travels rapidly to the muscle and on its arrival at the neuro-muscular junction excites the muscle fibers with but little delay. Bernstein (36) has shown that the interval between the arrival of the nerve impulse at the junction and the initiation of the propagated disturbance in the muscle fibers is about 3σ. Now in this record of Athanasiu the first alleged muscle response follows the first alleged nerve response by an interval of about 18σ. It was not until about 7 impulses had traversed the nerve that the muscle began to respond. No explanation is offered for this paradoxical failure of the muscle to respond in the usual way to the first six or seven motor-nerve impulses.

But aside from this inherent weakness in the argument it seems to us that our experiments make it clear that the nerve impulses involved in maintaining sustained contraction of reflex origin could not possibly be recorded in the electromyogram in the way Athanasiu contends. We have shown that even under the most favorable conditions, that is, with the nerve directly connected with leading-off electrodes arranged for monophasic recording, no appreciable excursion can be detected in most experiments during continuous discharge of motor impulses of central origin such as occur during sustained contraction; at most only minute excursions are recorded by this method. Even when the electron-tube amplifier is used there is generally no electric response of sufficient magnitude to be distinguishable from the slight unsteadiness of the base line which this instrument involves. With the leads applied to the innervated muscle conditions are far less favorable for recording the nerve impulses than when the leads are applied directly to the nerve; for the nerve trunk, being a tissue of high resistance, is surrounded by muscle fibers of comparatively low resistance which must necessarily serve to short-circuit the greater part of the action current in the nerve fibers and prevent their effective recording in the galvanometer. In order to demonstrate this point we have performed a series of control experiments in which we applied leads to a mammalian muscle, and stimulated it through the motor nerve. Having recorded the normal action current of the muscle we injected curare into a vein and as soon as muscular paralysis was complete we stimulated the

nerve again with a maximal shock, the leads still being in place on the muscle, in order to record whatever electric response could be detected from the branching nerve fibers within the muscle substance. Having done this we dissected out the nerve, applied leads to it directly, and recorded its maximal monophasic action current. The results of a typical experiment of this sort are shown in figure 14. The top record (A) shows the maximal action current of the nerve directly recorded. The next (B) shows the excursions obtained when the leads were on the muscle after it had been paralyzed by curare. It will be seen that small excursions are still visible. These are probably the action currents of the branching nerve fibers, for no trace of contraction could be detected in the muscle. And yet it is possible that a few muscle fibers were still responding. Therefore we may conclude that the maximal nerve trunk response with this

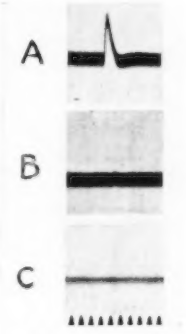


Fig. 14. Records to demonstrate the impossibility of recording action currents of nerve during sustained reflex when leading off from muscle.

A, Maximal monophasic action current of peroneal nerve; leads on nerve. B, Response of peroneal nerve to maximal stimulation; leads on curarized tibialis anticus muscle. C, Record from popliteal nerve during height of reflex response.

A, B, February 19, 1924. Hindle galvanometer; 17,000-ohm, gilded quartz string, 2.75μ diameter, tension 69 m. per amp. 0.2 microfarad condenser damping. Speed of film 12½ cm. per second. Magnification 490.

C, November 15, 1923. Hindle galvanometer; 16,800-ohm, gilded quartz string, 1.25μ diameter, tension 204 m. per amp. 0.2 microfarad condenser damping. Magnification 490.

condition of recording could be no larger than the excursion seen in B, and possibly would be even smaller. In C is shown a typical record made from the motor nerve during the crossed extension reflex. That is, a record of the central discharge in the nerve trunk recorded under the most favorable conditions. It will be seen here that there are no visible excursions. Obviously the action current of the nerve trunk during the reflex, as recorded by Athanasiu's method of leading off from the muscle, would be as much smaller than the excursions shown in C,—too small to detect,—as the excursions in B are smaller than those in A. This consideration should suffice to show the extreme improbability that the oscillations observed by Athanasiu were due to the action currents of nerve fibers.

The presence of a small electric response after the muscle has apparently been completely paralyzed by curare lends some support to a suggestion recently made (37, p. 607), that the discrepancy between the results of Lucas (38) and those of Samojloff (39) as to the delay in the response of a nerve-muscle preparation to the second of two stimuli might be explained by the appearance in Samojloff's records of the second action current in

the nerve fibers within the muscle. In order to illustrate the comparative sizes of the normal action current of muscle and that which we ascribe to the nerve endings in the muscle paralyzed with curare, we have reproduced in figure 15 typical examples of maximal responses in each case, from one of these experiments.

Henriques and Lindhard (40) have contended that muscle fibers do not give rise to action currents, and that the recorded electric response in muscle is due wholly to the motor nerve endings. If this were true then the great decrease in the size of the action current in our experiments, under the influence of curare, would signify the blocking of conduction between the nerve fibers and the supposed juctional tissue. But the recent work of Jinnaka and Azuma (41), together with certain considerations discussed in a recent communication from this laboratory (37, p. 603) has thrown considerable doubt on the existence of a special junctional tissue physiologically distinct from both nerve and muscle. Furthermore, Adrian and Owen (42) clearly showed the fallacy in the experiments and

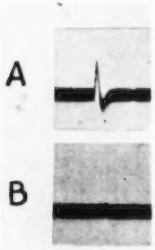


Fig. 15. Responses, before and after intravenous injections of curare, to maximal stimulation of peroneal nerve with leads on tibialis anticus muscle.

A, Normal response of muscle. B, Response of curarized muscle showing very small electric response. Records are from same experiment as figure 14, A and B.

argument of Henriques and Lindhard and refuted their contention. There is, therefore, no need to consider the response shown in figure 15 A, or indeed those in any of our experiments in which leads were applied to contracting muscles, as other than the action current of the muscle fibers themselves.

SUMMARY

1. The crossed extension reflex in the decerebrate animal has been studied by means of the electric response in the extensor muscles of the hind limb, and in the motor nerves supplying these muscles. It has been done both in the case of single stimuli and repeated stimuli of various frequencies. In most cases the presence of reflex response has been controlled at the same time by recording mechanical contraction. When the electric response of the muscle has been studied the mechanical record has been made from the same muscle. When the electric response of the nerve has been recorded, e.g., the popliteal, supplying the ankle extensors, the contraction of the knee extensors has been recorded, since these muscles normally contract synchronously with the ankle extensors.

We were obliged to rely thus on an allied muscle because, in order to get any response at all from the nerve, we recorded the action currents monophasically, which involved damaging the nerve, thus interrupting its continuity, and thereby paralyzing the innervated muscle.

2. We found great variation between different preparations in the crossed-extension reflex as observed mechanically, especially in the case of single stimuli. In some cases there was no visible response to a single stimulus, in others single stimuli evoked responses when the preparation was fresh, but failed to do so after the experiment had been in progress for some time.

3. In this reflex the electric response of the motor nerve to single stimuli was at most very small compared with the maximal response evoked by direct stimulation of the nerve itself. In the case of the sustained response to repeated stimulation we rarely could detect any response in the nerve at all. In one or two experiments small excursions, probably of physiological origin, were found correlated with the stimuli, but we cannot be absolutely certain that these were not due to artefacts. The usual disappearance of even the small electric response in the motor nerve resulting from single stimuli, as the discharge of motor nerve impulses increased and became more general (indicated by simultaneous record of mechanical contraction of the synergic muscle), is probably explained by the great brevity of the invididual nerve action currents and the fact that the individual responses in the many fibers of the nerve get completely out of phase with each other as the reflex progresses.

4. The electric responses of the gastrocnemius muscle are far easier to record than those of the motor nerve under conditions of reflex excitation. In the case of single stimuli the resulting response, when distinguishable from the background of decerebrate rigidity, appears normally as a brief tetanic response indicative of repetitive discharge of impulses from the center. When a sustained reflex is evoked by repeated stimulation we have in only one experiment found the correlation in frequency between the responses and the stimuli which Cooper and Adrian found in several of their preparations under apparently similar methods of observation. Our records almost invariably showed the irregularity in both frequency and amplitude of response characteristic of the voluntary electromyogram. We interpret this irregularity as depending on the perpetually varying number of muscle fibers getting in step with each other and responding for the time being synchronously. The observed frequency of responses depends on how small a group of individual action currents the recording apparatus is capable of detecting.

5. Although the electric responses recorded from the muscle in this reflex are much greater than those which appear in a corresponding nerve record, even in muscle the size of the individual response at the height of reflex activity is very small compared with the maximal action current

which can be recorded from the same muscle when a single maximal stimulus is applied to its motor nerve; yet at the same time the reflex mechanical effect is greater than that of the maximal twitch. This fact testifies to the high mechanical efficiency of sustained contraction as contrasted with that of the single twitch.

6. Although we find that after paralysis of a muscle by curare a small electric response can be detected in it upon stimulation of the motor nerve, probably due to the action current of the branching nerve fibers within the muscle substance, still the lack of any discernible action current under the more favorable condition of leading off directly from the motor nerve during the height of a sustained reflex response, renders it obvious that none of the oscillations recorded from contracting muscle by Athanasiu can reasonably be interpreted as due to the action current in the nerve fibers. The all-or-nothing law is assumed in this discussion, and certain objections to it which have been raised by other investigators are answered.

We wish to thank Miss Anne Hopkins for help in preparing the paper for publication.

BIBLIOGRAPHY

(1) Forbes and Gregg: This Journal, 1915, xxxvii, 118.
(2) Forbes and Gregg: This Journal, 1915, xxxix, 172.
(3) Forbes, Cobb and Cattell: This Journal, 1923, lxv, 30.
(4) Sherrington: Proc. Roy. Soc., B., 1921, 108.
(5) Adrian and Forbes: Journ. Physiol., 1922, lvi, 20.
(6) Sherrington: Integrative action of the nervous system. New York, 1906.
(7) Liddell and Sherrington: Proc. Roy. Soc., B., 1923, xcv, 299.
(8) Cooper and Adrian: Proc. Roy. Soc., B., 1924, xcvi, 243.
(9) Buytendyk: Zeitschr. f. Biol., 1912, lix, 36.
(10) Forbes and Thacher: This Journal, 1920, lii, 409.
(11) Forbes: This Journal, 1921, lvi, 273.
(12) Forbes: Quart. Journ. Exper. Physiol., 1912, v, 149.
(13) Liddell and Sherrington: Proc. Roy. Soc., B., 1924, xcvi, 212.
(14) Sherrington: Quart. Journ. Exper. Physiol., 1909, ii, 109.
(15) Sherrington: Journ. Physiol., 1894, xvii, 237.
(16) Gasser and Erlanger: This Journal, 1922, lxii, 516.
(17) Forbes and Rappleye: This Journal, 1916, xlii, 228.
(18) Forbes: Physiol. Rev., 1922, ii, 361.
(19) Sherrington: Journ. Physiol., 1910, xl, 28.
(20) Adrian and Olmsted: Journ. Physiol., 1922, lvi, 426.
(21) Whitaker and Forbes: This Journal, 1921, lv, 291.
(22) Piper: Elektrophysiologie menschlicher muskeln. Berlin, 1912, 97.
(23) Roaf: Quart. Journ. Exper. Physiol., 1912, v, 31.
(24) Bayliss: Livre Jubilaire du Prof. Ch. Richet, 1912, 471.
(25) Salamonson: Brain, 1920, xliii, 369.
(26) Sherrington: Brain, 1915, xxxviii, 203.
(27) Athanasiu: Journ. de Physiol. et de Path. Gen., 1923, xxi, 1.
(28) Gotch: Journ. Physiol., 1902, xxviii, 395.

(29) Lucas: Journ. Physiol., 1909, xxxviii, 113.
(30) Pratt: This Journal, 1917, xliii, 159; xliv, 517.
(31) Adrian: Journ. Physiol., 1914, xlvii, 460.
(32) Hill: Physiol. Rev., 1922, ii, 310.
(33) Fenn: Journ. Physiol., 1923, lviii, 200.
(34) Adrian: Arch. Nèer. de Physiol. de l'homme et des animaux, 1922, vii, 330.
(35) Lapicque: Compte Rend. Soc. de Biol., 1923, lxxxviii, 35.
(36) Bernstein: Arch. f. Physiol., 1882, 329.
(37) Forbes, Ray and Griffith: This Journal, 1923, lxvi, 553.
(38) Lucas: Journ. Physiol., 1910, xli, 368.
(39) Samojloff: Zentralbl. f. Physiol., 1910, xxiv, 45; Pflüger's Arch., 1912, cxliii, 453.
(40) Henriques and Lindhard: Arch. f. d. Ges. Physiol., 1920, clxxxiii, 1.
(41) Jinnaka and Azuma: Proc. Roy. Soc., B., 1922, xciv, 49.
(42) Adrian and Owen: Journ. Physiol., 1921, lv, 326.
(43) Forbes and Ray: This Journal, 1923, lxvi, 435.

STUDIES IN GASTRIC SECRETION

I. The Psychic Secretion of Gastric Juice under Hypnosis[1]

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Received for publication June 2, 1924

The purpose of this experimentation was to determine whether the gastric glands could be influenced by suggestion under hypnosis by causing the subject to suppose himself to be eating food when he was actually getting nothing to eat. It was early found that the gastric glands could be made to respond at will by suggestion in a susceptible subject. The results were confirmed by adequate controls, and the question then arose as to whether the gastric glands in man could be made to respond to suggestion in the waking state. The results in the latter case were also positive.

Literature. The available literature contains but few references to the susceptibility of the gastric glands to stimulation or inhibition in a subject previously hypnotized. In his experiments on sham feeding with bouillon, bread and milk, Heyer (1) noted an increased rate of secretion in his hypnotized subjects. Heyer (2) found that suggestion of pain and danger under hypnosis arrested at once the gastric secretion in nearly all his subjects. The suggestion of agreeable events (e.g., winning money in a lottery) had a similar effect. Bennett and Venables (3) found a marked inhibition of gastric secretion following the suggestion to a hypnotized army officer that he was flying in foggy weather and was forced to make a landing in enemy territory.

Methods. The subject in these experiments was a student of law, male, twenty-one years of age. The gastric juice was collected at ten-minute intervals previous to giving or suggesting meals, at fifteen-minute intervals following. Juice was aspirated by means of a modified Rehfuss tube as used in the Physiology Laboratory, University of Chicago. When test meals were given, just enough was aspirated to yield 2 or 3 cc. of filtered juice. One cubic centimeter portions diluted with 25 cc. of distilled water were titrated with N/40 NaOH solution using dimethylaminoazobenzene and phenolphthalein as indicators for the free and total acidities respectively.

[1] The expense of this research was defrayed by one of us (Dr. Arno B. Luckhardt).

RESULTS. *Control series 1: Ewald test meal given without hypnosis.* Figure 1 shows a curve of the free and total acidities constructed from an average of three experiments. Note that the fall in acidity due to the dilution with the test meal and the rise due to the response of the gastric glands form approximately a right angle.

Control series 2: Ewald test meal given under hypnosis. These control experiments were designed to determine what effect hypnosis would have on the acidities obtained by the actual feeding of the test meal. It is seen from these experiments that the fall in acidity due to dilution with the test meal and the rise due to the response of the gastric glands form an acute angle. In other words, the subsequent rise in acidity is more abrupt than in control series 1 (see fig. 2). Separate curves of the experiments showed no departure from this rule. A typical specimen curve is given in figure 3.

Series 3: Ewald test meal suggested under hypnosis but not given. The subject was told he was getting the test meal but was given an empty glass

TABLE 1

15 MINUTE INTERVAL	AVERAGE FREE ACIDITY	AVERAGE TOTAL ACIDITY	
No. 1	0.176	0.210	Before hypnosis
No. 2	0.163	0.201	Before hypnosis
No. 3	0.250	0.275	After induction hypnosis
No. 4	0.280	0.324	After induction hypnosis

In each of the five experiments from which this table was made hypnosis was induced immediately after the second fifteen-minute interval.

instead. He went through the usual motions of eating. In this group of experiments the first three samples were taken in the waking state. Following removal of the third sample hypnosis was quickly induced and the subject was forthwith caused to believe he was eating the test meal. All three graphs of free acidities are given in figure 4. In each case the rise in acidities did not occur until the second fifteen-minute sample. Since there is no dilution here there is no drop in acidity following the suggested meal. The question arose, why a more abrupt rise in acidity under hypnosis? The solution was found while a continuous secretion curve was being obtained under hypnosis, as a control, without suggesting food (fig. 5). This experiment indicates that the difference in rise of acidities in figures 1 and 2 is due to induction of hypnosis alone. The specimen experiment is quite typical. An average of five similar experiments gave the same results where hypnosis was induced especially to see what effect it alone would have on the secretion curve (see table 1).

At no matter what time during the course of an experiment and at no matter what level of acidity the stomach was then secreting, there was

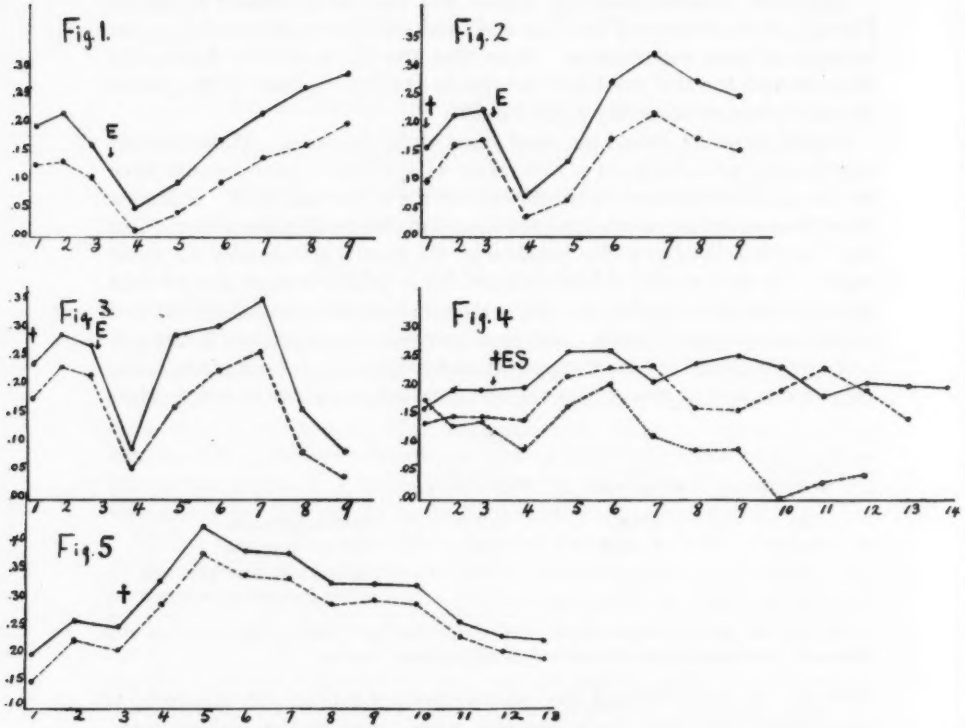

Fig. 1. Graph of Ewald test meal as control.

Fig. 2. Ewald test meal given under hypnosis. Figures 1 and 2 each are average graphs of three experiments.

Fig. 3. Single experiment of Ewald test meal given under hypnosis.

Fig. 4. Ewald test meal suggested to subject under hypnosis; free acidities of three experiments.

Fig. 5. Effect of hypnosis upon the continuous secretion curve; hypnosis induced five minutes following removal of specimen 3.

Explanation of figures: E, Ewald meal given; *ES*, Ewald meal suggested.

Obelisk, induction of hypnosis—in figure 1, hypnosis not induced; figures 2 and 3, induced before first specimen was taken; figure 4, induced after the third specimen was taken and meal immediately suggested thereafter.

In all the figures specimens 1 to 3 taken at ten-minute intervals, the remaining specimens at fifteen-minute intervals.

In figures 1, 2, 3 and 5: ———— is for total acidity, ---------- for free acidity. In figure 4 all lines are for free acidity.

Figures in the ordinates represent percentages of HCl; those in the abscissae represent the number of specimens of gastric juice taken.

in every instance an abrupt rise in acidity following the induction of hypnosis.

Whereas it was found as a rule that hypnosis alone had very little immediate effect on the volume of gastric juice secreted, the suggestion of food following the induction of hypnosis almost immediately doubled or trebled the previous rate just as it was found when suggesting food to the same man under appropriate conditions in the waking state. For example, in an experiment of obtaining continuous secretions while the subject, awake, was reading a book, the volumes ran as follows: 9.5 cc., 5.5, 5.5, 6.5, 9, for successive fifteen-minute intervals, respectively, a total of 36 cc. for one hour and a quarter. The subject was interrupted a moment to tell him he ought to eat a lot of prune juice frequently—the next five specimens yielded the following volumes, 12, 13, 12, 13 and 16 cc. respectively, a total of 66 cc. for an hour and a quarter.

In some instances peristent talking of food only slightly increased the volumes but markedly increased the acidities, while in other instances under apparently the same conditions talking of food, still in the waking state, nearly trebled the volume of the samples over those preceding the talking, the effect lasting strongly for some forty-five minutes.

The question arose, can a psychic secretion be elicited under hypnosis aside from the factor of removal of inhibition? Hypnosis was induced and the subject left asleep long enough for the spontaneous rise in acidity to subside, analyses being made at the usual intervals. This took varying periods of time, from one to two hours on different occasions. At the end of this time when the acidities had about reached their normal levels, the suggestion of food was given, and under these controlled conditions there was each time a rise of the acidities comparable with those obtained when food was actually taken in the waking state. Table 2 illustrates strikingly the results of suggestion of eating several articles of food supposedly more palatable, after the primary rise due to inhibition has subsided. The first rise in acidity was due to hypnosis alone, as in figure 5.

In another experiment this first rise in acidity was allowed to subside (time required, 1½ hours) and the Ewald meal again suggested. A rise in acidity resulted comparable to that in figure 4.

We shall now return to the question of palatability. The experiments show that if a test meal is suggested there is a progressive rise following an apparent latent period, this rise continuing 30 or 40 minutes, then gradually a dip downward and a secondary rise lasting as long as the first in some cases. With regard to the duration of time during which the acidity remained high, this curve (fig. 4) compares quite well with that obtained with the actual feeding of the test meal (fig. 3), both under hypnosis. Now, if instead of simply suggesting the test meal one continues to suggest the taking of a copious helping of palatable food every fifteen minutes, after

what would normally constitute a hearty meal has been suggested the subject says he has enough and cares for nothing else to eat—this in spite of the fact that he was very hungry at the outset but has actually had no food to eat. Instead of lasting nearly two hours following the suggestion of the last helping, as in some cases with the single Ewald meal, we find that when

TABLE 2

TIME	VOLUME	FREE ACID	TOTAL ACID	REMARKS
	cc.			
2:19 p.m.	12	0.036	0.072	Residuum clear
2:20	Hypnosis was induced in about one minute			
2:30	5.5	0.082	0.1185	Very tenacious
2:40	8.0	0.1641	0.1915	
2:55	5.5	0.1732	0.2097	
3:10	7.0	0.1732	0.2097	
3:25	8.0	0.1185	0.1641	
3:40	8.3	0.1185	0.1641	
3:55	6.5	0.1732	0.2097	
4:10	8.0	0.1185	0.1641	Bile stained
4:19	Suggested cream tomato soup. Simulated eating			
4:25	12	0.1550	0.2097	
4:27	Suggested steak, buttered toast, subject again simulated eating. Second helping desired by subject			
4:40	30	0.2280	0.2736	Considerably bile stained
4:42	Suggested creamed potatoes with gravy and toast. Subject went through motions of eating			
4:55	13	0.2553	0.2918	
4:57	Suggested mince pie and milk. Subject admitted that none like it was served in the Greek restaurants			
5:10	9	0.2097	0.2462	Slightly bile stained
5:25	9	0.2736	0.3098	Clear and viscous
5:45	13	0.2736	0.3098	
6:00	8	0.2280	0.2644	
6:15	6.5	0.1368	0.1824	Deeply bile stained
6:17	Subject awakened by suggestion			

this imagined satiety is reached the acidities may return to normal within thirty or forty-five minutes, so that this banquet curve remained up very little longer than that of the single test meal. (See fig. 4 and tables 2 and 3.) This may be explained on the ground that the test meal is agreeable to a hungry person and does not satisfy, whereas suggesting a complete banquet brings satiety and thus removes the psychic stimulation which seems to induce the appetite secretion.

When interpreting experiments which overlap in many respects we can find many points in one group which give strength to another group, but we must always be prepared to take into account the fact that just at the critical moment when we would expect a fall in acidity we may get a regurgitation of alkaline juices from the duodenum which would be responsible for the fall. This factor was controlled by a sufficient number of experiments. If we grant that a drop in acidity often means little, a marked rise

TABLE 3

TIME	VOLUME	FREE ACID	TOTAL ACID	
2:35	55.0	0.1368	0.2188	Residuum. Bile tinged
2:45	15.0	0.1368	0.2188	Slightly bile tinged
2:55	13.0	0.1641	0.2462	
3:00	.....Hypnosis induced			
3:05	Creamed tomato soup suggested; subject went through motions of eating and seemed to enjoy it			
3:10	23.0	0.1094	0.1824	
3:12	Hot, browned pork chops suggested, also bread with butter. Subject went through motions of eating			
3:25	27.0	0.2006	0.2918	
3:27	Creamed mashed potatoes with gravy, buttered toast and milk suggested. Subject simulated eating			
3:40	18.5	0.2188	0.3098	
3:42	Hot mince pie with milk. After going through motions of eating, subject stated he had had an immense meal and that his appetite was quite appeased			
3:55	18.0	0.2188	0.3098	
4:10	11.5	0.2553	0.3463	
4:25	6.5	0.2462	0.3372	
4:40	12.0	0.1368	0.2097	Bile tinged; very viscous
4:55	5.5	0.1094	0.1732	Bile tinged; very viscous
5:10	8.5	0.1185	0.1824	Bile tinged; more viscous
	Subject awakened			

in acidity immediately following a series of specimens of low acidity is significant.

The effect of suggesting a palatable meal under hypnosis without waiting for the spontaneous rise in acidity to subside. Table 3 is typical of experiments which show that suggestions of food immediately following the induction of hypnosis have the effect of temporarily checking the abrupt rise in acidities which would result from hypnosis alone. We have attributed the abrupt rise (fig. 5) resulting from the induction of the hypnotic state to removal of cortical inhibition. In such cases we found that acidity

began to fall within about thirty minutes, but when hypnosis was followed immediately by suggestion of food (table 3) the high acidity would persist for over an hour.

Psychic secretion obtained by verbal suggestion in the waking state. Many of these various experiments lasted over a period of three to five hours at

TABLE 4

TIME	VOLUME	FREE ACID	TOTAL ACID	
	cc.			
1:40	50.0	0.2097	0.2463	Residuum clear
1:50	13.0	0.2462	0.2827	Clear
2:00	6.5	0.1915	0.2371	Bile tinged
2:15	4.0	0.2371	0.2827	Clear
2:30	7.0	0.2097	0.2462	
2:45	9.5	0.2006	0.2280	
3:00	8.0	0.2006	0.2371	
3:15	9.0	0.2006	0.2371	
3:30	8.5	0.2188	0.2553	
3:45	9.0	0.2188	0.2644	
3:46	Talked eight minutes about appetizing food; subject awake			
4:00	25.5	0.2736	0.3098	
4:15	25.0	0.3190	0.3463	
4:30	13.0	0.3372	0.3737	
4:45	9.0	0.3098	0.3463	
5:00	4.5	0.3281	0.3646	
5:15	4.5	0.2736	0.3098	
5:30	6.0	0.2644	0.2918	
5:45	6.0	0.2644	0.2918	
6:00	12.0	0.2097	0.2371	
6:15	15.0	0.2097	0.2462	
6:20	Hypnosis induced. No other suggestions were given. Subject allowed to sleep quietly			
6:30	15.0	0.2644	0.3007	
6:45	8.0	0.3098	0.3463	Bile tinged
6:47	Subject given a glass of water with suggestion it would purge him. On drinking he soon complained of pain. He was awakened and had the desire to go to stool. However, the purge was without definite effect.			

the close of which time the gastric acidity was at a low level, as specimen curves show. Advantage was taken of this ideal condition at the close of long experiments to test the effect of verbal suggestion of food in the waking state. Results were gratifying. Two (more prolonged) experiments were then devoted to this phase in an effort better to analyze the results obtained under hypnosis. These experiments were performed in such a way as to give further strength to the work already done. Below a table is shown of the continuous secretion over a period of two hours while

the subject read a book. Suddenly the subject was interrupted and talked to for eight minutes about various appetizing foods, after which time he continued to read the same book. (See table 4).

From the above table it can be seen that suggestion in the waking state may cause a marked increase in volume as well as in acidity. The two hours following removal of residuum showed a yield of 74.5 cc., while the two hours following the suggestion of food, 3:46 to 5:45 p.m., showed a a yield of 105.5 cc. The effect of suggestion therefore lasted two hours, after which time hypnosis was induced. As usual there was a rise in acidity following the induction of hypnosis alone, as shown by the last two samples in table 4.

Another experiment showed that verbal stimuli repeated four times following withdrawal of samples of gastric juice apparently had no more lasting effect than when a single stimulus was given. This tallies quite well with previous experiments wherein we compared the effect of suggesting a number of varied articles of food under hypnosis and found that the suggestion of the single Ewald meal as a rule caused the acidity to be maintained at a high level just as long as when the articles of food suggested were more numerous.

Discussion. It has been mentioned that the rise in acidity (free and total) and sometimes volume following hypnosis alone is possibly due to removal of inhibition which the higher centers always maintain over the gastric secretory mechanism in the waking state. Apparently this inhibitory control reasserts itself partly after two to four hours following the induction of hypnosis.

The fact that we can get an additional marked rise in the volume and acidity of the gastric juice by suggestion alone, after the first rise due to removal of inhibition has subsided, is proof that the stimulation by suggestion alone is capable of producing a secretion curve comparable to that obtained by the administration of the test meal.

It is to be noted that other workers have failed to take account of the rise of acidity due to hypnosis alone although some of their graphs plainly show the phenomenon (3).

The relation between the palatability of the food and the response of psychic origin, of the gastric glands, is apparently dependent upon appetite. Under hypnosis the test meal suggested to a hungry man will produce a curve approximately of as long duration of high acditity as when food is suggested to the extent of satiety. This is probably explainable on the ground that when the single test meal is suggested the subject's appetite is still keen and serves to make the stimulation more effective. The sense of satiety, however, suggested in the hypnotic state inhibits the gastric secretory mechanism.

The fact that the gastric glands can be so readily influenced by suggestion in man in the waking state would indicate that our approach to man in psychotherapy is practically as great in the waking state as in the hypnotic state, so long as in either case the individual becomes a willing listener. In the waking state a man more nearly accepts what he considers as reason, and turns it into auto-suggestion before acting upon it. Under hypnosis a man may accept practically all that is said to him and afterwards will reject much which seems to him illogical when it comes to continued performance.

SUMMARY

1. The gastric glands respond more promptly to a test meal actually ingested when the subject is under hypnosis than when he is in the waking state.

2. Suggestion of a test meal under hypnosis causes a secretion curve with acidities equally high as when the test meal is actually given.

3. The induction of the hypnotic state itself results in a secretion curve as high in acidities as can be obtained by any other method.

4. Suggestion of food immediately after induction of hypnosis causes a delay in the spontaneous rise in acidity, due perhaps to the increased volume of juice, but the suggestion of food under hypnosis results in an increase of volume of gastric juice generally two or three times that of the control period before hypnosis.

5. Under hypnosis, after the spontaneous rise in acidity has subsided, suggestion of food causes an additional rise comparable with that obtained when the test meal is actually given.

6. Suggestion of food repeatedly to the extent of satiety results in a curve no higher in acidity and not appreciably longer in duration than that obtained when only the Ewald test meal is suggested (comparisons made under hypnosis).

7. The talking of food in the waking state caused a psychic secretion, but with the time interval used (15 minutes) there was no latent period between the stimulation and the rise in acidity. The volumes were also increased in this case.

BIBLIOGRAPHY

(1) HEYER: Kongress der Deutsch. Gesell. f. inn. Med., Wiesbaden, 18-21, April, 1921; abstract in Therap. d. Gegenw., Berlin, 1921, lxii (23), 268.

(2) HEYER: Therap. d. Gegenw., 1921, 62 (23), 285; also Arch. f. Verdauungs-Krankheiten, Berlin, 1921, (dependent on abstract—original not accessible).

(3) BENNETT AND VENABLES: Brit. Med. Journ., 1920, ii, 662.

STUDIES ON INTESTINAL INHIBITORY REFLEXES

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Received for publication June 18, 1924

The literature contains abundant evidence that both the motor and secretory activities of the stomach are sharply influenced by nerve impulses originating at points distant from that organ. The work of Pavlov (1) bears mainly on the secretory activity, while that of Carlson (2), more recent, deals with both the motor and the secretory. Many others have contributed to this phase of physiology. Less work of this type has been done on the intestine, with the result that our knowledge of reflex effects on the intestine is less complete and satisfactory.

Surgeons and laboratory workers well know the relative quiescence of the gastro-intestinal tract after opening the abdomen. While factors such as temperature changes, loss of carbon dioxide and drying may be important, yet the general opinion is that the inhibition is largely reflex, due to impulses originating at points irritated by instruments or by handling of the viscera. The disappearance of some types of enterospasm after merely opening the abdomen and lightly handling the intestines is an illustration of this point.

One also meets cases of pelvic irritation with which there is associated nausea, vomiting and often constipation. In many instances these disturbances cannot well be explained on the basis of a toxemia, particularly if one adheres strictly to the known facts and findings. It is believed by many that these phenomena are often purely reflex.

A number of specific and definite observations, in addition to those mentioned in the opening paragraph, bearing in gastro-intestinal reflexes, have appeared in the literature. Carlson, Pearcy and Boyd (3) have demonstrated changes in the tonus of the cardia associated with stimulation of various points in the abdomen and pelvis. White (4) reported that irritant injections into the colon delay the emptying of the stomach. Hotz (5) in making a study of the reflex excitability of the intestinal tract in peritonitis observed inhibition of the intestines following stimulation of the urinary bladder.

In our own laboratory, about a year ago, during an observation on the effects of intravenous injection of sodium bicarbonate on duodenal motility, a record was obtained bearing directly on this question. During a period of marked activity on part of the duodenum the animal micturated.

The duodenum relaxed promptly and all movement ceased for a few seconds. After the urine had been passed, the intestine rapidly regained its tonus and movements of the original type set in. We have observed similar instances since that time in the students' laboratory.

We later undertook to extend this observation, and the following report is based upon the data obtained. We have succeeded in demonstrating that the movements of the small intestines in the dog may be inhibited by impulses originating in the urinary bladder, rectum, peritoneum, and from some points on the surface of the body.

Dogs were used throughout. On the whole we were not able to note any difference between sexes in the groups listed as favorable animals. With the bladder work we were successful with a larger percentage of males than females. Dribbling of the urine occurred more often in the females, and this, as will be shown in more detail later, usually wrecked the experiment. The presence or absence of food had no marked effect on the results. Large dogs were more satisfactory than small ones. The superiority of the larger animals for intestinal work was pointed out by us in a previous communication dealing with observations on villi movements (6). The exact reason for this difference is not clear, but possibly the shock produced in preparing the smaller animal is proportionately greater.

The choice of anesthetic is also of paramount importance, because the animals must be maintained in a state of good reflex excitability. Ether proved uniformly unsatisfactory. Barbital, 0.3 gram per kilo body weight given by stomach tube, proved quite satisfactory. A few of the animals were partially decerebrated.

The intestinal records were all made with a balloon tambour system. The balloons were made of thin rubber finger cots. Air and water transmission was used. A pressure bottle was connected with the tube leading from the balloon to the tambour through a T tube. The balloon end of the system was filled with water, and the tambour end with air. During the experiments the pressure within the system was maintained at about 35 cm. of water.

A study of the reflex effects on the movements of the duodenum from stimuli originating in the urinary tract was first undertaken.

Figures 1 and 2 are records taken from the same animal at about a ten-hour interval. The animal was given barbital at 8:15 in the morning, and prepared for the experiment about an hour later. Three hundred cubic centimeters of warm saline were given intravenously. The urinary bladder was fairly full to begin with, but after the injection of the saline filled rapidly, and emptied itself at 9:40 a.m. The duodenum manifested good motility from the start. It will be noted that immediately following the micturition act the intestine lost some tonus but rapidly regained it. Several such records were taken during the day with similar results. At

7 p.m. another injection of saline was made. At 8 p.m. the animal again micturated. Figure 2 is a reproduction of the record made at this time. The animal was in excellent condition, but the tone of the intestine had diminished somewhat, and the movements were almost purely segmental. It will be noted that after the emptying of the bladder the tonus of the intestine fell sharply, and movements almost ceased. Recovery gradually took place.

It would appear from the above findings that the mere filling of the bladder, or better, bladder pressure when brought on slowly does not to any marked degree affect the motility of the duodenum. In order to test out this point a cannula was put into the bladder through the apex. The cannula was connected with a pressure bottle which could be raised or

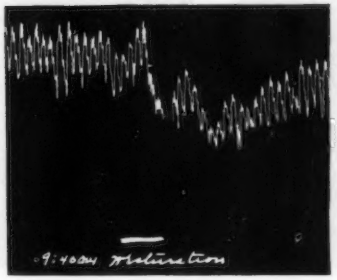

Fig. 1

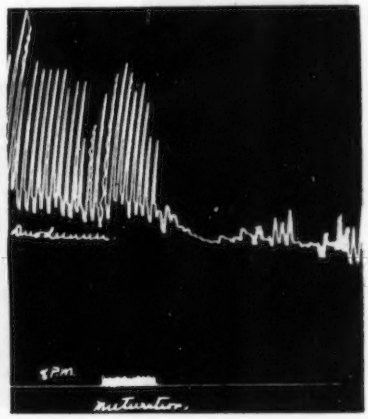

Fig. 2

Fig. 1. Relaxation of the duodenum of the dog during micturition.

Fig. 2. Relaxation of the duodenum of the dog during and following micturition. Taken from the same animal as the record in figure 1, about ten hours later.

lowered at will. The fluids used in the pressure system at different times were saline, tap water and dog urine. We were not able to detect any difference between the effects produced by these different fluids. In case the pressure bottle was raised very slowly no change in duodenal activity took place which could not be interpreted as normal. The response to sudden and rapid increase in pressure was somewhat variable, but quantitative rather than qualitative. We never observed any augmentation of duodenal tonus and movements under these conditions, but quite constantly a diminution. In many cases the relaxation was small, in others

very prompt and complete. Figure 3 in a record of such a complete response. Figure 4 is more representative of the usual result. It will be noted that the sudden increase in pressure was followed by a slight diminution of duodenal tonus, and that when micturition set in the diminution in tonus and movement was more rapid and complete. These results indicate that the main source of afferent impulses responsible for the duodenal reflex is not from the bladder wall, but from some other part of the urinary tract, possibly the urethra.

Bearing on this point also are observations which we repeatedly made, to the effect that neither pinching the bladder wall nor electrical stimulation produce any marked or constant effect on the activity of the duodenum.

Fig. 3

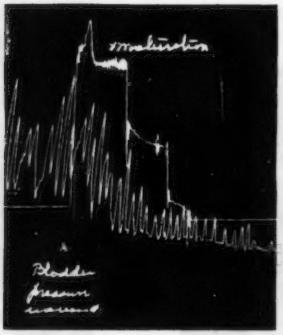

Fig. 4

Fig. 5

Fig. 3. Effect on duodenal motility a of rapid increase of pressure within the urinary bladder. At *X*, pressure bottle raised, at *U* the animal began to micturate, and at *Y* the pressure bottle was lowered.

Fig. 4. Effect of increasing urinary bladder pressure on duodenal movements. At *X* the pressure bottle was raised. At *Y* the animal began to micturate.

Fig. 5. The effect of catheterization on duodenal movements. Male dog. The catheter was rapidly pushed into the proximal urethra.

Impulses from the urethra were studied through the agency of the catheterization. Both male and female dogs were used. For the female dogs small glass catheters were used, for the male small soft rubber. The females in this instance responded more sharply than the males. In the males very little effect was recorded until the catheter reached the proximal urethra. This part was quite sensitive. Figure 5 is a record taken from a male dog. The duodenal tonus before the catheterization began was good and held quite evenly. On insertion of the catheter there

was some fluctuation in tonus. On reaching the proximal urethra the fall in tonus was sharp, and remained low for about 45 seconds. The return to normal was quite rapid in spite of the fact that the catheter was left in place. In two female animals showing good response, ice cold saline, saline at body temperature, and a 0.5 per cent hydrochloric acid solution in saline also at body temperature were injected into the urethra, the nozzle of the catheter being about one centimeter from the bladder orifice. The warm saline was the least effective, and the acid solution the most. The same amounts were injected each time, and at the same rapidity as nearly as possible. In both of these animals the catheters were pushed into the bladder, and the warm and cold salines tried, this before the use of the acid. The results in both animals were much less marked than when the fluid did not reach the bladder. In this instance the cold saline was more effective than the warm. In order to obviate and temperature stimulation of the urethra these experiments were repeated but the fluid was introduced by means of a needle through the apex of the bladder. The results were similar to those following the first procedure. Not enough fluid was put into the bladder to distend it to any marked degree.

These results would indicate that the duodenal inhibition following bladder distention and micturition is due mainly to impulses from the mucosa of the proximal urethra. The mucosa of the bladder is not nearly so sensitive. Impulses from the wall of the bladder may play a small part in the reaction.

We next turned our attention to the effect of impulses coming from the colon and rectum. After considerable "watchful waiting" and patience we succeeded in obtaining a few records of the effect of defecation on various levels of the small intestine. These movements were spontaneous in the sense that no artificial stimulus was employed to bring them about. Figure 6 is a record from the ileum taken during defecation. It will be noted that there was a sharp diminution in tonus which lasted for only a short time. Recovery was very rapid. Several such records were taken from the ileum, and several from the duodenum and jejunum. One was taken from an animal with a jejunal fistula which had been established some weeks before. In this case no anesthesia was used. In no instance were the effects so marked as in the ileum. While the number of records is hardly large enough to enable us to draw definite conclusions, yet on basis of what we have it would seem that the effects of defecation are less pronounced the higher up the small intestine one goes.

The next step taken was to study this reflex through the agency of defecation brought about by warm soap enemata. The colon and rectum were filled with the fluid through a colon tube.

We learned almost immediately that the stimulation of the anal canal and rectum on insertion of the tube to a slight degree modifies the movements of the small intestine. The outstanding effect was that of a diminution in tonus, but in a number of animals this was preceded by just a momentary increase in tonus and movement. In the majority of animals defecation was brought on after about 150 cc. of the enema had been introduced. The effects on the movements of the various segments of the small intestine were in no way different from those associated with spontaneous defecation. Occasionally an animal was found which did not retain the

Fig. 6

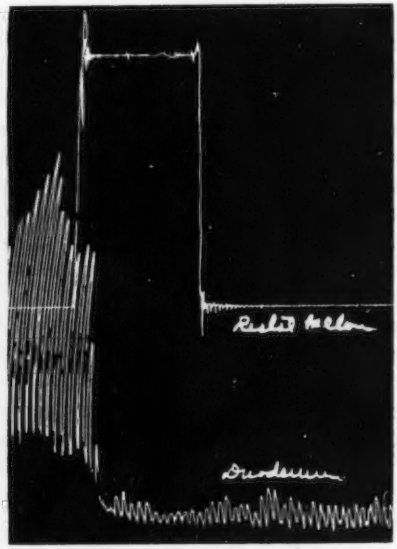

Fig. 7

Fig. 6. Inhibition of the ileum during defecation.

Fig. 7. The effect on the duodenum of sudden and sustained stimulation of the rectum.

enema well. In these instances the effects on the small intestine were irregular and much less pronounced. These results indicated that while stimulation of the rectal mucosa is a factor, yet it appeared that a stretching of the wall of the large intestine is of greater significance.

We undertook to throw light on this last possibility by inserting a balloon into the rectum and connecting it with a pressure bottle. In this way we were able to more sharply localize the stimulus, and also to maintain any pressure desirable.

The results in this series were uniformly clear. Figure 7 is a record taken from the duodenum of an animal into the rectum of which a balloon had been placed. It will be noted that when the pressure was increased in the balloon the duodenum promptly relaxed. The balloon used was a thin finger cot. The pressure record was taken with a mercury manometer. The pressure recorded in this tracing is just a little below the bursting point of the balloon. The results are the sharpest when the pressure is suddenly increased. In many of our tests where the pressure was gradually increased the effects on the small intestine were only slight and in some cases negligible. In this group another phenomenon came into more prominence, that of a preliminary augmentation of movement in the small intestine. This was by no means constant, yet it occurred often enough to make it stand out in contrast with its almost complete absence in cases when the stimulus was rapidly made intense.

We next turned our attention to the effects of stimulation of the peritoneum. The relative quiescence of the intestines immediately after opening the abdomen has already been mentioned. In order to establish conditions favorable the animal was allowed to rest after the preparation for making records, the abdomen being closed. The peritoneum was stimulated mechanically, using a sharp pointed and curved probe. In some instances the probe was put into position at the time of the initial preparation, in others it was inserted through a small opening left in the path of the incision. We were not able to note any difference in the results obtained from these two procedures.

Figure 8 is a record taken from the jejunum, the line of peritoneal stimulation being just a little above the level of the umbilicus. There was a brief augmentation of movement, followed by a marked diminution in tonus and movement. All parts of the small intestine responded in a similar fashion, but a comparison of the records taken from the different levels suggests that there is somewhat of a segmental innervation, that is to say, the duodenum responds more sharply to stimulation of the peritoneum near the costal margin than in the pelvic region. Our observations were extended to the large intestine, but the results were unsatisfactory. We were not able to obtain regular and sustained movements of any part of the large intestine without artificial stimulation, neither were the results constant in those instances where we obtained movements. In some animals no changes were recorded on stimulation of various peritoneal regions, although the small intestine responded sharply, in some there was some diminution in tonus and movements, and in others augmentation. Either the large intestine is not so much influenced reflexly as the small, from the peritoneum at least, or the conditions of our experiments did not allow us to observe such effects. Further and more extensive work is required to clear up this point.

An observation of practical value in work of this kind may not be out of place here. Naturally in all physiological work one is ambitious to approximate natural and normal conditions. In some of our animals we were not able to obtain good movements of the intestine. In these animals, after several hours of waiting we usually resorted to artificial stimulation, such as the injection of sodium bicarbonate, warm saline and physostigmin salicylate. This procedure scarcely ever proved worth the effort, for in only a few instances were we able to obtain the inhibitory reaction which has been so outstanding in the experiments described above. Possibly the motor stimulus was so overwhelming that the more feeble inhibitory impulses made but little if any impression. One cannot help but think of end results as depending upon factor equilibria. It is possible and highly probable that many of the stimuli used in obtaining the reactions already described were way out of proportion to any that may occur in the normal living animal. Of the artificial methods used for stimulating the small

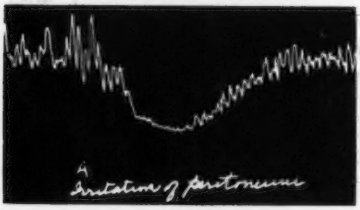

Fig. 8

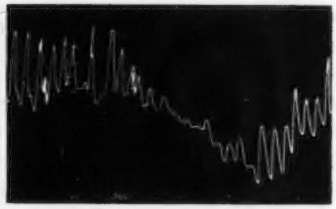

Fig. 9

Fig. 8. Record from the jejunum. At *A* the peritoneum was stimulated mechanically.

Fig. 9. Record taken from the jejunum of a dog through a fistula which had been made some weeks before. At *X* the skin was pinced. No anesthesia.

intestine, saline deserves special mention. In the animals in which the small intestine was refractory the intravenous injection of from 300 to 500 cc. of warm saline in almost every case brought about an improvement in tonus and movement. This effect was very marked in some. We have occasionally seen this observation in the literature, but is scarcely ever mentioned prominently. Because of its possible practical value, and because it is a verification of the observation of others it seemed worth while to call attention to it.

So far the outstanding observations are that during micturition and during defecation the tonus and movements of the small intestine are diminished, and that this reaction can to some extent be brought about by artificial stimulation of the urinary bladder, urethra and rectum. It has also been shown that stimulation of the peritoneum brings about intestinal inhibition. One would naturally assume that this reaction is brought

about through a reflex arc, the efferent element being the splanchnics. One might think of the possibility of impulse conduction through the intrinsic nervous mechanism of the intestine. In the so called peristaltic reflex a wave of inhibition spreads from the point of stimulation, and as has been shown by various observers this wave may travel in either direction. This remote possibility cannot apply directly in case of bladder or peritoneal stimulation, and is not orthodox even if the stimulus is applied to some remote part of the intestine.

This last point is easily disposed of, inasmuch as we found that complete section of the intestine between the point of stimulation and the point of record did not modify the reaction.

In order to throw further light on the nervous mechanism involved records were taken from animals both before and after section of the splanchnics. In a large number of the animals so handled we were not able to obtain satisfactory movements of the intestines after the nerve section, but in the small fraction which came through in good condition we found that complete splanchnic section completely abolished the inhibitory reaction following bladder, urethral, rectal and peritoneal stimulation.

Our attention was next turned to the vagi. So far the evidence would indicate that the vagi are little if any involved in the reaction. The thought suggested itself that both vagi and splanchnics might be involved with the splanchnics predominating. If such were the case then one would expect to record augmented movement on stimulation of the normally effective points. The results on the animals with the splanchnics sectioned show that this is not the case. On reversing the situation and sectioning the vagi there is no accentuation of the inhibitory reflex, at least the change is so slight that one cannot be sure of it. The only evidence that we have had at any time pointing to a possible vagus factor is that of an occasional brief preliminary augmentation, which was mentioned in the earlier paragraphs of this paper.

The only drug employed to modify the reflex excitability in our animals was strychnine. We used it occasionally in some of the refractory animals for the purpose of improving their condition. It did not prove a brilliant success, but in a few of the animals there was a decided improvement in intestinal tonus and movement following the giving of the drug. The drug was used on other occasions for the purpose of increasing the excitability in animals already showing good reflex activity with the idea that we might be able to exaggerate the reflex effect on the intestine. The drug was given in doses sufficient to cause the animal to respond slightly to a quick but light tap on the tip of the nose. The drug did not modify the general character of the results, but on the whole the records show a slight increase in the reflex effects. In this connection it should be noted that Carlson and Luckhardt (7) in a study of skeletal reflexes brought about by stimulation of visceral afferents found that strychnine increased the skeletal responses.

In another series of animals the hypogastric nerves were stimulated. As a result we uniformly registered a diminution in the tonus of the various segments of the small intestine under observation. But never so marked as when the rectum or urethra were stimulated. It would appear that only a part of the afferent path is in these nerves.

The last series of experiments dealt with the effects of stimulation of various points on the periphery of the body. The first of this group of observations was made on a day when the heating plant failed to cope successfully with the temperature factor in our laboratory. Between tests the animal was kept covered to prevent heat loss. In order to carry out some procedure we uncovered the lower part of the body. A cold draft was blowing in through a ventilator just to the left of the animal. The duodenum relaxed very abruptly. The animal was again covered, and the intestine rapidly regained its tonus. The accident was so suggestive that the procedure was deliberately carried out a number of times with similar results. We found that uncovering the upper part of the body, leaving the lower covered, had very little effect. That cooling was the factor responsible was borne out by the fact that the application of cold water and ice produced the same result, but water at body temperature did not. This result led to other types of skin stimulation. The effect of pinching was tested out. The only spots to which there was any definite response were of the lower abdomen, perineum and the tip of the nose. We never succeeded in getting any response from the back. These results were verified on the two intestinal fistula dogs which were observed without the use of anesthesia. Figure 9 is a record taken from one of these animals obtained while massaging and pinching the skin of the abdomen around the fistula which opened to the outside about an inch below the umbilicus and about an inch to the right of the midline.

Discussion. The observations described above point clearly to the fact that the tonus and motility of the intestinal tract are reflexly affected by impulses originating in distant parts of the body. The predominating effect is that of inhibition. We cannot say that stimuli arising from these points never augment intestinal activity, because the conditions under which the experiments were done were in the majority of cases somewhat artificial. The conditions, however, in the animals from which records were taken during spontaneous defecation and micturition, as well as in the animals with the intestinal fistulae were nearly enough normal to justify the conclusion that inhibitory effects are brought about during the normal life activities. In the light of this the intestine appears to react in a fashion analogous to that of the stomach.

By carrying the results into the fields of clinical experience, as well as into the experiences of daily life, it seems justifiable to say that facts have been brought out which strengthen "reflex" explanations. One is

reminded of the chain of gastrointestinal symptoms associated with pelvic disturbances, with rectal irritations, and with irritations of the bladder and urethra. One often observes marked relief from certain forms of intestinal colic after giving a warm enema, and this often occurs even if the enema is returned practically clear. It is well known that pressure on the anus helps to control and delay the defecation reflex. It is commonly taught that in many cases hemorrhoids develop as the result of constipation, and it is a well-known fact that constipation is commonly associated with them. Our results would suggest that after the tumors are formed, the constant irritation due to them may tend to establish a vicious cycle. Finally we are reminded of the restlessness and various skin sensations, such as chills up and down the back, associated with an urgent desire to micturate. While a part of this complex may be psychic, yet it is suggested that the chills, at least, are due to pure vasomotor reflexes. We attempted to study this condition in the dog by recording blood pressure during the filling of the bladder, and during micturition. The results were not entirely conclusive. The blood pressure curve showed some unevenness, but this may have been due to the restlessness of the animal. We never recorded enough of a rise in blood pressure to suggest any extensive vasoconstriction. We do not know, however, just what change in blood pressure takes place along with the vasoconstriction necessary to give a chill sensation.

SUMMARY

1. Evidence has been presented showing that impulses from the urinary tract, rectum, peritoneum and from certain skin areas reflexly diminish the tonus and movements of the small intestine.

2. The splanchnics contain the efferent paths for these reflexes.

3. The vagi are very little, if any, involved in these reactions.

4. Some of the afferent paths involved are in the hypogastrics.

5. The facts presented are interpreted as throwing light on the mechanism involved in producing some of the disturbances involving the gastrointestinal tract, associated with irritating conditions in the pelvis, and also as explaining some of the phenomena accompanying and following micturition.

BIBLIOGRAPHY

(1) Pavlov: The work of the digestive glands. London, 1910.
(2) Carlson: The control of hunger in health and disease. Chicago, 1916.
(3) Carlson, Pearcy and Boyd: Arch. Int. Med., 1922, xxx, 409.
(4) White: Quoted by Sollmann: Manual of pharmacology, 1922, 217.
(5) Hotz: Mitteil. a.d. Grenzgebiet. d. Med. und Chir., 1909, xx, 257.
(6) King and Arnold: This Journal, 1922, lix, 97.
(7) Carlson and Luckhardt: This Journal, 1921, lv, 366.

SOME OBSERVATIONS ON THE EFFECT OF INCREASED AIR MOVEMENT AND WATER INTAKE ON THE DOG DURING AN EXPOSURE TO HIGH ENVIRONMENTAL TEMPERATURE[1]

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Received for publication June 21, 1924

Some time ago Flinn and Scott (1) discussed the results of experiments on dogs exposed to high environmental temperatures, with the purpose of applying the knowledge gained from these experiments to the general effects observed among furnace workers. They found that at the environmental temperatures of 45° and 50°C. the body temperature of the dog rises so rapidly and to such a height that it seemed to them unwise to expose the animal to these temperatures for a longer period than one hour. They also observed a concentration of the blood due to the excessive evaporation of body water; an increased oxygen capacity, which could be explained by the concentration of the blood; a slightly increased oxygen content of the blood, due to an increased lung ventilation; a rapid fall in carbon-dioxide content and capacity; an increased pH (alkalosis), due to an excessive pulmonary ventilation, with a concomitant washing out of the carbon dioxide, and without a compensatory loss of alkali from the blood. At 45° no change in the blood sugar was noted during the one-hour exposure, but at 50°C. there was a sharp rise. The dogs were exhausted after the exposure, and the blood conditions did not return to normal until several hours had elapsed after removal from the heat closet. The occurrence of high body temperature followed by a subnormal temperature was indicative of heat exhaustion. These conditions, together with alkalosis and apparent exhaustion of the animal, would indicate that the animal was in a serious condition—at least temporarily.

While studying the conditions of workers in the glass industry, data were collected on the pulse rate, the blood pressure and the body temperature of men working in front of the glory-holes of the glass furnace, where the average temperature in the location of the gatherer was 50°C. Records were kept of the condition of the men both immediately before they began to work and immediately afterwards. These observations were taken daily over a period of two weeks, and showed no special change in the

[1] Approved for publication by the Surgeon General.

physical measurements during the daily exposure. The pulse and body temperature remained normal, while there was a very slight drop in the blood pressure. The men worked on four-hour shifts, and apparently were no more tired than it was normal for a man to be after a day's work in a cooler location.

A glance at the results reported by Flinn and Scott will show that temperatures similar to those endured by furnace workers affect dogs much more severely than they do men. It will be noted, however, that the attendant conditions under which the dogs were exposed were correspondingly more trying. Thus, in furnace work there is considerable air movement, produced by either a plenum system of fans, while the dogs were denied almost all air movement except the little amount necessary to prevent a temperature stratification, estimated to be about fifty feet a minute. Again, furnace workers drink an abundance of water during their exposure to high temperatures, while the dogs were denied any water during the course of the experiment. It was therefore thought desirable to continue the research reported by Flinn and Scott by subjecting the dogs to conditions which would more nearly simulate those borne by the men. This paper is the report of such a series of experiments.

In carrying out the present research, it was decided to divide it into two series. It one series the effect of an increased air movement alone was noted, and in the other the effect of an increased air movement and water was studied. In the experiments carried out by Flinn and Scott, the air movement was approximately 50 feet a minute; in this study it was increased to 224 feet a minute. This air movement was created by means of electric fans.

In the second series of this research, water, heated to 30°C., was given the dog from time to time; it was at first poured down the dog's throat, but as a rule the animal soon learned to drink the water offered it in a pan held in front of it. Water was given every twenty minutes, so as to duplicate more nearly the condition of the furnace worker. It was found impracticable to leave the pan of water in the closet with the animal, because it would spill the water in attempting to get into the pan for the purpose of lying in it and producing what might be called an artificial sweat. It cannot be said that we duplicated the exact condition of the worker, because he had the benefit of cold water to aid him in keeping his temperature down. Our object in giving the dog warm water was to see whether or not a replacement of the body water was required rather than a lowering of the body temperature by absorption of the calories necessary to raise the temperature of the water from 10° or 12° to the temperature of the body. We realize that we were not quite faithful to our plan in heating the water to only 30°C. The dogs varied in the amount of water consumed, but this averaged 2 or 3 liters each experiment.

Procedure. It was considered sufficient in these two series of experiments to continue the examination of the blood as to oxygen content and capacity, carbon dioxide content and capacity, blood sugar and total solids. The body temperature and weight were observed each time that blood was drawn for analysis.

Physical. The temperature chamber, method of control and means of preventing stratification and pockets of air were the same as those described by Flinn and Scott. The relative humidity within the chamber was determined by the sling psychrometer; and the air movement, by means of a Short and Mason anemometer, readings being taken in various parts of the chamber.

The body temperature was taken with a clinical thermometer by rectum, the thermometer being left in place for a minimum of a minute and a half.

Chemical. Carbon dioxide and oxygen contents and capacity of the blood were determined by the method of Van Slyke and Stadie (2), using the short form of the Van Slyke apparatus, which is considered of sufficient accuracy for comparison work.

The blood sugar was determined by the Hastings and Hopping (3) modification of the MacLean (4) method.

The total solids. This determination was made in duplicate on 1 cc. of blood dried to constant weight in silicon crucibles at 110°C. in an electric oven.

Biological. No change was made in the method of feeding and handling the dogs from that described by Flinn and Scott, so as not to influence the results. A rest period of at least two weeks was permitted each dog between experiments, to allow for recovery from any deleterious effect of the high temperature.

The need of this rest period after an exposure to high environmental temperature has been clearly demonstrated by our own experience in the loss of animals from attempting to cut down the time between exposures when only physical measurements were being made and before we realized that some change had taken place in the animal during the previous exposure from which it had not yet recovered. In each case the animal was apparently in normal condition as far as temperature, blood analysis and behavior would indicate. The change referred to may possibly be a modification of the nervous system, since Goldschneider and Flatau (5) have shown that the nerve cells in the ventral horn undergo a change in their structure after an artificial heating of the animal in an environmental temperature of 42 to 44°C. Barker (6) calls attention to the fact that when the animal has been removed from such environmental temperatures, there is a gradual restitution of these cells, but that the rate of repair is not nearly so rapid as the appearance of the function would indicate, and that complete recovery requires at least several days. Halliburton and

Mott (7) have shown that well-marked changes occur in the Nissl's granules and that the nerve cells will coagulate if a temperature of 42°C. be maintained for some time. Shefford (8), in his work on evaporation, calls attention to the fact that short exposures to a high rate of evaporation increase the sensibility to evaporation on the succeeding exposure.

The dogs in the present series of experiments were restless during the first part of the exposure to an environmental temperature of 50°C. with an air movement of fifty feet per minute. They made efforts to escape from the cage, if confined to it, or, if tied, they gnawed at the woodwork of the closet in an endeavor to escape. In half an hour this period of excitability passed and was followed by one of semi-indifference to their surroundings, and they appeared at times to be on the threshold of coma. At the end of the hour-exposure it was necessary to lift them out of the closet, since they made no effort to move by themselves. This period of indifference lasted for the greater part of an hour after their removal from the heat, but at the end of two hours they appeared to have recovered their faculties.

Other investigators have called attention to this period of excitability in animals which is apparently inherent to a rise in body temperature during an exposure to high temperatures. Sutton (9) has noted the same signs in man when there is a definite rise in the rectal temperature to 99.5°F. (37.5°C), at which point there would appear to be an abrupt change from sleepiness to wakefulness and irritation. If, under continued exposure, the rectal temperature should rise to 101–102°F. (38.36–38.9°C.), a man could no longer carry on sustained mental activities, such as reading a book or learning a vocabulary. As the rectal temperature increases to 103°F. (39.1°C.), any irritation is very trying to the temper. Sutton considers that this irritability is closely associated with the rise in body temperatures and that it is a warning of an impending exhaustion of the central nervous system.

When the air movement was increased to 224 feet a minute during an exposure of 50°C., the animals, while perhaps more restless than at lower temperatures, remained quiet during the first four hours of exposure, though there did not seem to be the same tendency to sleep as at the lower temperatures. This non-appearance of excitability during the entire four hours is rather hard to explain, in view of the foregoing remarks, since after the first hour there was a definite rise in body temperature, though not to the same heights as with the lower air movement.

The explanation which seems most probable to us grows out of our own experience in the heat closet. With the lesser air movement, the heat seemed unbearable after the exposure had continued some minutes. On the other hand, when the air movement was increased, as in the second series of experiments, there was a pleasant sense of coolness, due to the

rapid evaporation taking place, and it was hard to realize that the dry bulb temperature was as high as in the first experiments.

During a discussion as to these observations, the suggestion has been made that possibly the critical body temperature lies between that obtained with an air movement of 50 feet a minute and that at 224 feet. A longer exposure to the air movement of 224 feet a minute might raise it to the body temperature obtained with an air movement of 50 feet. If so, would the dog become restless? We can only say that in the cases in which we lost our animals the period of restlessness was not observed.

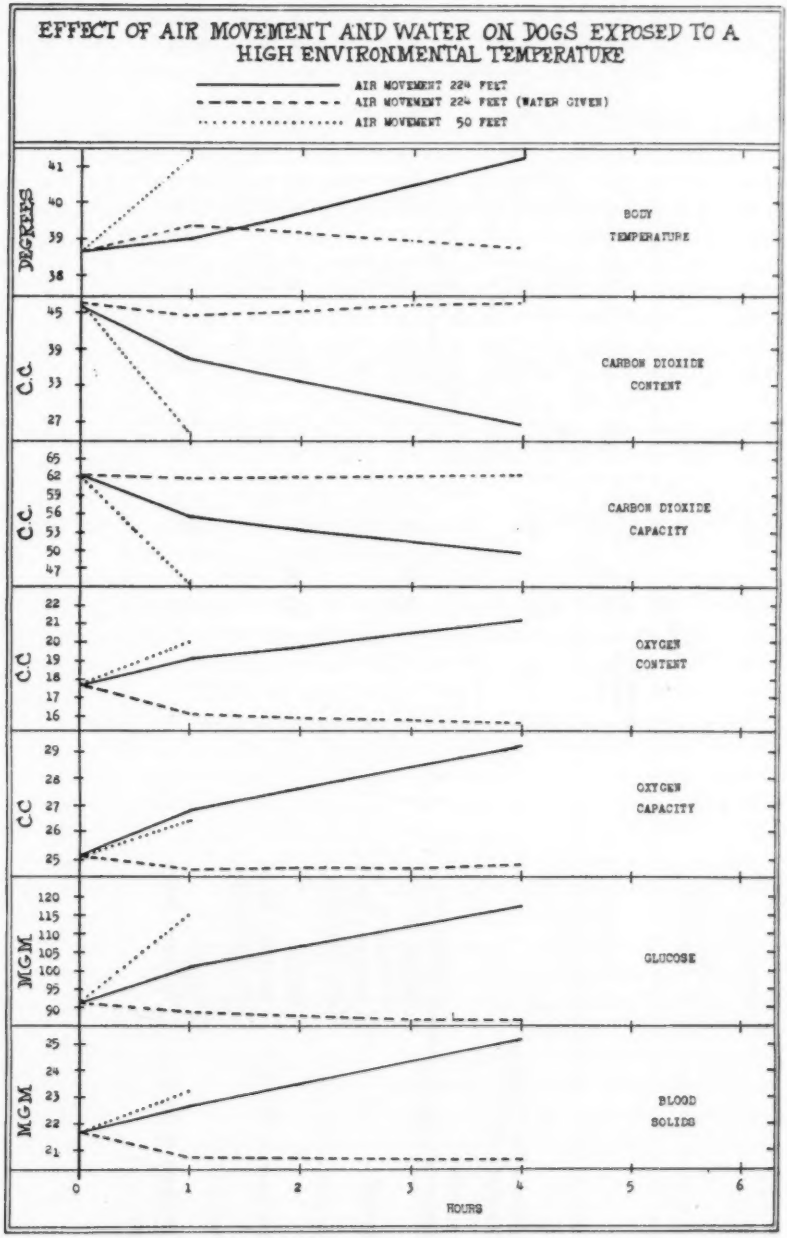
The animals seemed fatigued after the exposure to an air movement of 224 feet and 50°C., and lay down quietly for a short time after removal from the heat closet. Recovery from the exposure, however, was fairly rapid, if their behavior could be taken as an indication, except in one instance. In this case the dog died within thirty hours after removal from the heat closet. It was unable to stand when removed from the heat closet and seemed partially paralyzed in the hind limbs. It refused all food, drank little water, and remained in a dazed condition until its death. Other dogs had lost as much water by weight, but this dog was small, and the loss amounted to 10 per cent of its body weight, which Hill (10) feels is the limit of loss for man.

In the series of experiments in which the dogs were permitted to drink all the water they desired during an exposure to an environmental temperature of 50°C. with an air movement of 224 feet, there was only a short period of restlessness before they had taken their first drink. Water was set before them every twenty minutes, and they remained quiet during the entire four hours, except for the period noted. They showed no apparent sign of fatigue when removed from the closet, and from all appearances were as fresh as when first brought into the laboratory.

Samples. All blood samples were drawn from the jugular vein by venepuncture.

For the convenience of the reader the results of our experiment are presented in the form of graphs. We used the same mean average condition of the animal as a base line that Flinn and Scott had used. This was done by adding algebraically to the means of the experimental series an amount sufficient to make the initial values of that series equal to the mean of all the corresponding initial determinations, as arrived at by them from 47 observations.

EXPERIMENTAL RESULTS AND DISCUSSION: *The effect of increased air movement and water intake on the body temperature during exposure to high environmental temperatures.* When exposed to an environmental temperature of 50°C. and an air movement of 50 feet, the dogs show a very marked rise in the rectal temperature, and apparently this rise begins at



(1) REMOVED FROM HEAT CHAMBER

TABLE 1

		NUMBER OF OBSERVATIONS	BODY WEIGHT	RECTAL TEMPERATURE	CO_2 CONTENT	CO_2 CAPACITY	O_2 CONTENT	O_2 CAPACITY	RED POWER AS GLUCOSE	TOTAL SOLID
			kgm.	*°C.*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*mgm. per cent*	*per cent*
Mean initial values		47		38.7	46.7	62.5	17.7	25.1	91	21.7
Standard deviation					±4.8	±4.9	±3.4	±3.1	±11	±2.1
1st series					Air movement 50 ft. per mm.					
Temperature of the chamber 50°C.					Relative humidity 28 per cent					
Initial	Mean		14.44	38.9	48.7	57.8	14.7	22.7	85	20.3
	Correction			0.2	2.0	4.7	3.0	2.4	6	1.4
	Modified Mean			38.7	46.7	62.5	17.7	25.1	91	21.7
	St. Deviation				3.1	2.6	2.2	3.0	7	2.5
1st hour in chamber	Mean		14.08	41.8	27.1	39.9	16.9	24.0	108	21.8
	Modified Mean			41.6	25.1	44.6	19.9	26.4	114	23.2
	St. Deviation				2.4	3.5	2.3	2.6	8	2.4
2nd hour of recovery	Mean			38.1	46.6	55.5	15.6	22.5	81	20.5
	Modified Mean			37.9	44.6	60.2	18.6	24.9	87	21.9
					5.0	5.0	2.8	2.8	10	2.5

TABLE 2

		NUMBER OF OBSERVATIONS	BODY WEIGHT	RECTAL TEMPERATURE	CO_2 CONTENT	CO_2 CAPACITY	O_2 CONTENT	O_2 CAPACITY	RED POWER AS GLUCOSE	TOTAL SOLID
			kgm.	*°C.*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*vol. per cent*	*mgm. per cent*	*per cent*
2nd series					Air movement 224 ft. per mm.					
Temperature of the chamber 50°C.					Relative humidity 33 per cent					
Initial	Mean		13.4	38.8	45.6	55.8	11.7	19.6	90	18.1
	Correction			−0.1	+1.1	+6.7	+6.0	+5.5	+1	+3.6
	Modified Mean			38.7	46.1	62.5	17.7	25.1	91	21.7
	St. Deviation				±2.7	±3.1	±6.1	±5.2	±8.3	±1.3
1st hour in chamber	Mean		13.6	39.2	36.4	48.8	13.4	21.3	100	19.10
	Modified Mean			39.1	37.5	55.5	19.0	26.8	101	22.70
	St. Deviation				±3.5	±5.5	±6.1	±6.6	±8.4	±2.4
4th hour in chamber	Mean		13.0	41.5	25.8	43.1	15.1	23.6	116	21.6
	Modified Mean			41.4	26.9	49.8	21.1	29.1	117	25.2
	St. Deviation				±2.1	±6.9	±5.2	±8.32	±7.8	±3.9
3rd series					Air movement 224 ft. per mm.					
Temperature of chamber 50°C.					Water given. Relative humidity 33 per cent					
Initial	Mean		12.6	38.7	41.3	52.7	15.7	20.10	98	20.9
	Correction				5.4	9.8	2.0	5.0	7	0.8
	Modified Mean			38.7	46.7	62.5	17.7	25.1	91	21.7
	St. Deviation				±2.6	±5.1	±4.6	±4.0	±4.2	±1.8
1st hour in chamber	Mean		12.8	39.4	39.1	51.9	14.6	19.6	95	19.5
	Modified Mean			39.4	44.5	61.7	16.0	24.6	88	20.8
	St. Deviation				±3.7	±4.6	±2.9	±3.9	6.8	1.3
4th hour in chamber	Mean		12.7	38.8	41.2	52.3	13.5	19.8	92	19.8
	Modified Mean			38.8	46.6	62.1	15.5	24.8	85	20.6
	St. Deviation				+3.4	+4.5	+3.5	+3.8	+8.6	+1.2

once. In fact, this rise was so sharp and reached such a height that it did not seem wise to expose the animal for a longer period than one hour.

When the air movement was increased to 224 feet a minute in the second series of experiments, at an air temperature of 50°C., the body temperature showed very little increase during the first hour, amounting to only 0.4°C. By the end of the four-hour exposure, it had risen to 41.3°C., an increase of 2.6°C. over the normal. This increase in body temperature in spite of the increased air movement is interesting in view of the fact that so much has been written lately on air movement. There seems no doubt, if we interpret our results correctly, that air movement must be studied carefully from the viewpoint of evaporation. Air movement may delay the discomfort of the organism exposed to high environmental temperature, by keeping the body temporarily cool because of the increased rate of evaporation, but at the expense of the water reserve of the organism. That the animal will survive in excellent condition on exposure to high air temperature or movement if the water lost is replaced concurrently, is shown by our second series of experiments. In this series, the animals were encouraged to drink all the water that they desired. During the first hour of exposure in this series there was a rise in body temperature of 0.7°C.,—a fact which is hard to explain, since we had the same air movement and temperature as in the previous series. There may be present in the body water which, in the normal animal, is freely available for temperature control. Water may also be present in excess of the actual needs of the animal, and it is only after this mobile water has been used up that trouble arises and extra water is required for both temperature control and the normal activity of the protoplasm. Whatever the explanation, it is not serious, in view of the fact that at the end of the four-hour exposure the animal's temperature was back to what it was at the beginning of the experiment.

The effect of air movement and water intake on the oxygen content and upon the amount of hemoglobin in the blood. With an air movement of only 50 feet a minute and an environmental temperature of 50°C., the increase of the oxygen capacity of the blood was commensurate with the concentration of the blood. When the air movement in the chamber is increased to 224 feet a minute, the oxygen capacity continues to increase with the increase in blood concentration. When water is freely drunk, we note that the oxygen capacity decreases as the blood solids fall with the replacement of the water lost by the blood because of evaporation.

The rate of respiration increases and becomes progressively more shallow as the environmental temperature increases with the low air movement. And though this increased breathing may result in an increased cooling efficiency, it does not apparently show a commensurate increase in the aeration of the blood,—probably because of its shallowness, the evaporation being largely from the mucous membranes of the mouth and trachea.

With the increased air movement, we do not get the labored breathing that is so noticeable with the same air temperature but lower air movement. Instead of a respiration rate of almost four hundred, we get one that is similar to that observed in a dog exposed to the sun on a hot summer day, deep and fairly rapid. The oxygen content increases as it did with the lesser air movement.

In the series in which water was freely drunk, the oxygen content dropped from the beginning, as the oxygen capacity did. There has been a growing impression in our minds that in many of our experiments the oxygen content, as well as the oxygen capacity, followed the course of the blood solids, and that the increase or decrease of content is due simply to an increased or decreased amount of hemoglobin, brought about by a concentration of the blood by the gain or loss of water for any given percentage of hemoglobin saturation.

Effect of increased air movement and water intake on the carbon dioxide content and capacity of the blood. The tachypnea resulting from an exposure to a high environmental temperature and a low air movement causes a lowering of the carbon dioxide capacity of the blood in an attempt on the part of the organism to compensate for the washing out of the carbon dioxide through the forced breathing.

With the increased air movement, depletion of the carbon dioxide capacity is more gradual, extending over the entire four-hour period. At the end of the four hours it had practically reached the low level attained in only one hour with the rapid breathing so characteristic of the lesser air movement. This slower depletion is directly related to the difference in the rate and type of breathing.

As would be expected from the other results discussed, when water was freely drunk, the alkali remained unchanged during the entire period of exposure. The curve of the carbon dioxide content of the blood presents the same picture as the alkali reserve, following directly the rate and type of breathing.

The relation between the concentration of the sugar of the blood and increased air movement and water intake at high temperature. Flinn and Scott related the rise in the sugar of the blood during an exposure to 50°C. of only one hour, which did not appear at 45°C. within a similar period, principally to the environmental temperature, and not exclusively to the body temperature or emotional disturbances accompanying the exposure to high temperature. That it was apparently not due to the concentration of the blood would seem to be indicated from a study of their curves. The concentration of the blood after an hour's exposure to 45°C. was apparently of the same degree as that at 50°C., and yet the blood sugar at 45°C. did not seem to be increased. The body temperature for both exposures is the same. Neither were there sufficient grounds for assigning the rise in sugar

to emotional stress. At an air movement of 50 feet a minute and environmental temperature of 50°C. the animal made frantic efforts at first to escape; while, on the other hand, when the air movement was increased to 224 feet a minute, the animal showed very little excitement. And yet the blood sugar rose practically to the same concentration at the end of a four-hour exposure to an air movement of 224 feet a minute as it did after an hour exposure to an air movement of 50 feet a minute.

The relation between the total solids of the blood and an increased air movement and water intake at high environmental temperature. With an increased air movement at the environmental temperature of 50°C., the blood solids increased in proportion to the rate of evaporation. It seems that the rate of dehydration of the body at this exposure is so rapid that the water-regulating mechanism is affected to such an extent that it cannot respond to the demands of the organism. But when water becomes readily available through a large intake, the blood is kept at the normal concentration, and no increase in blood solids is found.

General results. The greatest source of danger from heat exposure appears to lie in the organism itself. The defense rests apparently on a good heart and vasomotor mechanism for the flushing of the skin and the maintaining of a sufficient blood pressure, both venous and arterial. Any disarrangement of the water-regulating mechanism is bound to result in a serious condition. Once the body temperature rises above its critical point, we find an increased general metabolism in accordance with chemical laws. The effect of this is a vicious circle of increased body temperature, resulting in an increased metabolism, followed by a further increased body temperature, and so on. Once this cycle has been initiated, there seems to to be no means of stopping it, unless the animal is removed to a more favorable environment, and water is made available to the organism either through releasing the body water which seems to have become bound through some chemical physical reaction, or by an intake of water through the anus or the mouth.

The great storehouse of the body water, according to Engel (11), is the muscles, which contain, from his observation, two-thirds of the total amount of water in the body. The affinity of the cell for fluid determines the retention of fluid by the cell. If, by any interference with the proper oxygenation of the tissue, the colloid becomes abnormally acid, this will increase the affinity of the cell for water.

The results of this research indicate that an increased air movement may prove temporarily beneficial to the organism if the exposure is not too prolonged. It appears to delay the rise in body temperature until that point is reached at which the water-regulating mechanism can not longer keep pace with the increased rate of evaporation. The main benefit of this increased air movement lies in the fact that it is constantly changing the immediate

layer of saturated air that surrounds the body and thus prevents evaporation. According to the laws of physical equilibrium, the pulmonary and evaporation increases with the degree of dryness of the atmosphere. It becomes almost double when there are 5 grams of water vapor instead of 9 in 1 c. m. of air. In short, the value of the elimination of water vapor of the organism varies inversely with the hydrometric state. It would of necessity follow that the higher the relative humidity of the air, the larger must be the volume of circulating air. Haldane (12) has shown that a man would stand a wet bulb temperature of 34.4°C. without any abnormal rise in rectal temperature, providing there was an air movement of 170 linear feet a minute.

The benefit of drinking water freely while engaged in any occupation necessitating an exposure to abnormally high temperatures is very apparent. The animals that we lost showed a high blood concentration. This can be referred to the fact that the water loss was large in proportion to their body weight. It would appear from this observation that the important fact may be not that the blood has become more concentrated, but that the body tissues must have lost a dangerous amount of their water.

At this critical point the muscles which, according to Engel (11), furnish 68 per cent of the water lost, can no longer function readily in the manner necessary to keep the blood volume constant. According to Durig (15), the muscles may lose as much as 10 to 20 per cent of their water without harm, yet a slight desiccation of the blood will impair the circulation. Any impairment of the circulation may effect functional disturbances in almost every part of the body. If the impairment of the circulation reaches the point where it results in a diminution in the pressure and volume of the venous stream, it may bring about cardiac failure as the immediate cause of death.

It is to be noted that water is most abundant in those tissues that are undergoing the most rapid metabolic changes and which are called upon to function most frequently and rapidly in a chemical rather than a structural manner. A lowering of the water content of the cell below the optimum amount will seriously interfere with its metabolic reactions and may result in the death of the cell, if the loss is not replaced rapidly. Northwag (13), working with the tissues of starved birds, came to the conclusion that when death did occur from lack of water, it was due to an accumulation of split products in the cell because of a lack of sufficient water to remove them. Shelford (8), in his observation on evaporation, reports most of the symptoms associated with heat exposure. These reactions were produced whether the evaporation was due to dryness of the air, air movement, or heat. Hill (10) estimates that a man loses 4.8 per cent of his body weight in the twenty-four hours of a summer day, and that if he is working hard, his loss may amount to 7.7 per cent. Hunt (14) estimates that a man

needs 1500 cc. of water a day to satisfy the urine and feces requirements and to neutralize by evaporation the heat added by evaporation metabolism, up to six liters. Hill repeatedly warns against a water loss of more than 10 per cent of the body weight.

SUMMARY

1. The body temperature rises gradually during the four-hour exposure to an environmental temperature of 55°C. and air movement of 224 feet a minute. At the end of four hours it rises to the same height as was obtained at the end of one hour's exposure with an air movement of 50 feet a minute and the same environmental temperature.

When water was freely drunk during the four-hour exposure, the body temperature remained normal, except for a slight temporary rise during the first hour.

2. The oxygen capacity of the blood follows very closely the concentration of the blood.

3. The oxygen content of the blood varies with the type of aeration taking place because of the exposure, but is not in direct proportion to the increased air movement over the membrane of the mouth and throat. The oxygen content bears a close relation to the hemoglobin concentration of the blood.

4. With an air movement of 224 feet a minute and an environmental temperature of 55°C. there is a gradual depletion of the alkali reserve of the blood from the beginning of the exposure and continuing throughout the four hours' exposure.

When water is drunk freely during the exposure, there is practically no change in the alkali reserve of the blood during the four-hour exposure.

5. The carbon dioxide content of the blood follows the trend of the alkali reserve during the exposure.

6. The concentration of blood sugar increases gradually throughout the entire four-hour exposure to an environmental temperature of 50°C. and an air movement of 224 feet per mm. When water is drunk during this exposure there is a slight drop in the blood sugar concentration.

7. Because of the higher rate of evaporation the percentage of blood solids increased rapidly from the beginning of an exposure to an air movement of 224 feet a minute and temperature of 50°C. At the end of one hour the degree of concentration was the same as was obtained at the end of one hour's exposure to an air movementof 50 feet a minute.

When water was freely drunk, the blood solids showed a tendency to drop slightly during the four-hour exposure.

8. An increased air movement benefits the organism by delaying the deleterious effects, but apparently at the expense of the organism itself.

9. The free drinking of water during an exposure to high air temperature is of greatest benefit in maintaining the organism in a normal condition.

BIBLIOGRAPHY

(1) FLINN AND SCOTT: This Journal, 1923, lxvi, 191.
(2) VAN SLYKE AND STADIE: Journ. Biol. Chem., 1921, xlix, 1.
(3) HASTINGS AND HOPPING: Proc. Soc. Exper. Biol. and Med., 1923, xx, 254.
(4) MACLEAN: Biochem. Journ., 1919, xiii, 135.
(5) GOLDSCHNEIDER AND FLATAU: Forschr. Med., 1897, 609.
(6) BARKER: The nervous system, 302.
(7) HALLIBURTON AND MOTT: Arch. Neurol., 1903, i[1].
(8) SHELFORD: Biol. Bull., 1913, xxv, no. 2.
(9) SUTTON: Journ. Path. and Bact., 1909, xiii, 62.
(10) HILL: Recent advances in physiology and biology. Chap. vii–xxi.
(11) ENGEL: Arch f. Exper. Path. u. Pharm., 1904, li, 346.
(12) HALDANE: Journ. Hyg., 1905, v, 496.
(13) NORTHWAG: Arch. f. Hyg., xiv, 273, 337.
(14) HUNT: Journ. Hyg., 1912, xxii, 470.
(15) DURIG: Pflüger's Arch., 1901, lxxxv, 401.

THE EFFECT OF THYROXIN AND ITS ACETYL DERIVATIVE ON AMPHIBIANS AND MAMMALS

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Received for publication June 23, 1924

The primary object of the experiments recorded here was to test whether or not there exists a fundamental difference between mammals and amphibians in regard to their physiologic responses to thyroid and iodine administration. Certain experiments of Kendall (1) and Swingle (2), (3), (4), (5) indicated that differences existed between these two vertebrate groups with respect to the chemical grouping in the molecule responsible for the changes induced in each, following thyroid administration. For instance, iodotyrosin, iodoserumalbumin, iodoserumglobulin and iodocasein, when fed to thyroidless or pituitaryless amphibian larvae, bring about a rapid and precocious metamorphosis, the effect produced upon these organisms being similar to those induced by thyroid feeding except that the metamorphic changes occur somewhat less rapidly. The same substances exert no influence upon amphibian transformation without the iodine in the molecule, nor can other halogens such as bromine be substituted for the iodine and the substances still retain their activity. However, it is pretty well established that iodized proteins and amino acid such as iodotyrosin, iodocasein, iodoserumalbumin and globulin exhibit no demonstrable effects when fed to mammals suffering from cretinism or myxedema and do not, when tested on normal animals, induce those physiologic responses which usually follow thyroid administration in this group.

Oswald (6) has studied the physiological properties of several iodized proteins and compared them with thyroid. He found, for example, that iodocasein, iodotyrosin and iodoserumalbumin are inactive when administered to mammals and do not exert thyroid-like effects.

Hellin (7) reported years ago that iodized albumin and nucleo-albumin prepared from the spleen are inactive when given to mammals, and Blum (8) in the year following publication of Hellen's paper reported that iodized albumin has practically no effects upon metabolism.

Strouse and Voegtlin (9) failed to observe any thyroid-like effects on the nitrogen metabolism or on the blood pressure of normal dogs, nor

was there any favorable effect on the condition of myxedematous and cretinous mammals following administration of diiodotyrosin.

The following experiment of Kendall (10) is an excellent illustration of the point involved, namely, that the substances inducing thyroid-like effects, i.e., metamorphic changes in amphibia have no influence upon the metabolic rate of mammals, and that the two classes of vertebrates probably react to quite different chemical groups in the thyroid hormone.

Kendall observed that injections of pure thyroxin into mammals are followed by a very definite and marked physiologic response. But when the hydrogen of the imino group in the thyroxin is replaced by acetyl, the substance loses its physiologic activity and there follows no demonstrable effect upon the metabolic rate. This emphasizes the importance of the imino group in thyroxin (insofar as the metabolic effect upon mammals is concerned) and minimizes the importance of iodine in the molecule. Bearing in mind the effects of certain organic iodine compounds upon tadpole metamorphosis, Kendall tested the acetyl derivative of thyroxin on tadpoles, for if the metamorphosis depends only upon the increase in the basal metabolic rate of the larvae then thyroxin should increase the rate of metamorphosis, but the acetyl derivative involving the imino group should not. If, however, iodine alone is concerned in accelerating metamorphosis, then both thyroxin and the derivative should affect the transformation. It was found that both thyroxin and the acetyl derivative would induce a rapid metamorphosis of the bullfrog tadpole. The conclusion was that thyroxin appears to have two separate and distinct functions: the effect upon the mammalian metabolic rate which is brought about by the CO-NH groups within the molecule; and the physiological changes involved in the metamorphosis of the tadpole due to iodine contained in the molecule.

Kendall's experiment is of great interest to workers on amphibian metamorphosis but, so far as we are aware, has never been published except in an abstract of about two hundred and fifty words, and all details are lacking. We have repeated the experiment on an extensive scale using normal men for mammalian material, and thyroidectomized and hypophysectomized tadpoles for the metamorphosis phase of the problem. In the course of the work interesting data relating to the physiological activity of thyroxin when taken orally were obtained.

We take this opportunity of expressing our obligation to Dr. E. C. Kendall of the Mayo Clinic for acetylating the thyroxin.

EXPERIMENTAL. *A. Amphibians.* Several hundred Rana sylvatica embryos were thyroidectomized and hypophysectomized, and kept until several months after the period of metamorphosis normal for this species had elapsed, and until all of the normal unoperated control animals from the same egg clusters had transformed into frogs. It will be recalled that

thyroidless and pituitaryless frog tadpoles do not metamorphose but permanently retain their larval characters. Sixty operated animals, thirty thyroidless and thirty pituitaryless, were injected intraperitoneally with small quantities of acetyl thyroxin, and a like number injected with an equal amount of pure thyroxin. An effort was made to study quantitatively the effects of thyroxin and its acetyl derivative when administered to tadpoles, but unfortunately small amounts of the injected solutions were in most cases lost following withdrawal of the needle from the peritoneal cavity or subcutaneous lymph sinuses. This was true despite the fact the animals were first anesthetized in chloretone solution. Owing to the loss of minute quantities of the injected substances, efforts to obtain exact quantitative data on the physiologic activity of the thyroxin and acetyl thyroxin were abandoned. Qualtitatively, however, the two substances behaved alike; both thyroidless and pituitaryless larvae responded to the injection by very rapid and precocious metamorphosis, the larger the amount given, the more rapid the transformation. We were able to induce marked metamorphic changes, such as development of the fore and hind limbs, tail resorption and development of the typical frog mouth, in thyroidless and hypophysectomized tadpoles, within nine days from the date of first administering the thyroxin and its acetyl derivative. Merely feeding a small amount of crystals of both substances to tadpoles was sufficient to induce very marked metamorphic responses. Several animals had well-developed fore legs (generally only the left one through the operculum) within five days of the date of first feeding. The rate of metamorphic change induced was so rapid that the animals invariably died before tail resorption was completed and in many cases before both fore legs appeared. The reaction was comparable in every way to that following the administration of thyroid extract or tissue.

Our experiments on administration of thyroxin and its acetyl derivative to thyroidectomized and hypophysectomized tadpoles confirm the observations of Kendall (10) who performed a similar experiment on normal bullfrog larvae, possessing intact thyroid and pituitary glands. It is probable, though our experiments do not prove it, that quantitatively thyroxin is somewhat more potent in inducing the metamorphic response of tadpoles than is the acetyl derivative. In a personal communication to one of the writers (W. W. S.), Kendall stated that his experiments showed that pure thyroxin is somewhat more active than the acetyl derivative in bringing about metamorphic change in tadpoles. However, there can be no question about the potency of acetyl thyroxin as an accelerator of anuran transformation for it is a highly active metamorphosis-inducing agent. Owing to lack of sufficient material we have not tested acetyl thyroxin on urodele larvae.

The second step in the work was to test the physiological activity of thyroxin and its acetyl derivative upon mammals. Both of the substances had proven to be active on amphibians but according to theory only thyroxin should show physiological activity when administered to mammals.

B. Mammals. Four adult, healthy, male individuals were used in the experiments, consisting of the writers and Mr. K. Donahue, Technical

TABLE 1

Record of pulse and general condition of a 33-year old male individual following an intravenous injection of 10 mgm. of thyroxin

DATE 1924	PULSE RATE			REMARKS
	4–6 p.m., highest	7:00 a.m., lowest	Average	
March 27	80	72	78	
March 28	80	70	78	
March 29	82	66	79	
March 30	84	72	80	
March 31	80	70	79	
April 1	84	70	80	Weight, 142 lbs. 10 mgm. thyroxin taken intravenously
April 2	112	84	104	Violent headache, dizziness, diarrhea
April 3	104	94	100	Headache, dizziness, muscular weakness
April 4	110	101	104	Symptoms same as before. Shortness of breath following slight exertion. Dizziness
April 5	108	100	104	Headache disappeared. Muscular weakness marked. Feel ill
April 6	104	95	100	Headache again. Dizziness. Muscular weakness
April 7	115	90	100	Headache and dizziness disappeared. Muscular weakness marked
April 8	109	92	97	Symptoms abating. Feel better
April 9	100	88	94	Weight, 140 lbs. Symptoms disappeared
April 10	102	88	94	
April 11	94	80	90	
April 12	90	80	87	Weight, 141.5 lbs.
April 13	88	77	85	
April 14	89	78	84	

Assistant in the Osborn Zoölogical Laboratory. A fifth man was employed in part of the work involving oral administration of thyroxin but after taking 54 mgm. he contracted severe tonsilitis and was not utilized further in the experiments.

We were not in a position to measure basal metabolism or in fact to make any kind of metabolic tests, and throughout were forced to rely entirely upon the pulse rate, nervous symptoms and variations in body weight indicating physiological response upon the part of the organism

to the administered thyroxin. This is a legitimate procedure and has been employed by Carlson (11) and Kendall (1) in testing the response of the individual to thyroid products.

According to Kendall (1) thyroxin, even in small amounts, will produce toxic effects. The severity of the toxic symptoms following administration of Alpha iodine (thyroxin) to normal individuals varies greatly, but in general is as follows: If to a normal individual with a pulse-rate of about 75 and a weight of about 75 kilos, 3 mgm. of thyroxin is given per

TABLE 2

Record of pulse and general condition of a 21-year old male individual following an intravenous injection of 10 mgm. of thyroxin

DATE 1924	PULSE RATE			REMARKS
	4–6 p.m., highest	7:00 a.m., lowest	Average	
March 28	85	59	70	
March 29	79	61	68	
March 30	82	61	70	
March 31	80	56	69	
April 1	84	59	71	Weight, 175 lbs. Took 10 mgm. thyroxin intravenously
April 2	98	74	86	
April 3	120	83	105	Muscular weakness. Dizziness, headache and diarrhea
April 4	94	89	92	General weakness and headache
April 5	86	74	82	Headache and dizziness
April 6	92	78	88	Muscular weakness
April 7	125	78	100	
April 8	121	83	100	Ill in bed
April 9	100	88	96	Muscular weakness. Out of breath following slight exertion
April 10	105	80	97	
April 11	99	81	95	Symptoms disappeared
April 12	101	78	90	Weight, 174 lbs.

day, on the day following the first dose there is no apparent change except a slightly increased pulse rate. If the same daily dose is continued the patient slowly reacts and in the course of eight or ten days, nervous symptoms develop, the pulse rate is around 130 to 140, there is a tendency to perspire more freely on exertion, and the individual may be short of breath and easily tired. At first there is increased appetite, but later loss of appetite, nausea and sometimes diarrhea develops. During this time a loss of weight varying from 1 to 5 kilos may occur, though this is not invariable. If smaller doses are given, the severity and course of symptoms are modified, but the patient will react to very small amounts of thyroxin. If larger doses than 3 mgm. are given, there results a serious condition

in which the above symptoms are exaggerated. Kendall states that some individuals develop severe symptoms following administration of as little as $\frac{1}{75}$ to $\frac{1}{25}$ of 1 mgm. thyroxin per body weight per day.

The thyroxin and its acetyl derivative were given orally and intravenously and to our surprise the thyroxin proved active only when given by vein. Tables 1 and 2 are records of the pulse rate and general condi-

TABLE 3

Record of pulse and general condition of a 33-year old male individual following an intravenous injection of 10 mgm. of acetyl thyroxin

DATE 1924	PULSE RATE			REMARKS
	4–6 p.m., highest	7:00 a.m., lowest	Average	
May 6	72	74	76	
May 7	79	66	77	
May 8	80	65	78	
May 9	81	68	77	
May 10	80	70	78	
May 11	78	67	76	
May 12	80	70	78	
May 13	80	70	78	10 mgm. of acetyl thyroxin taken intravenously at 4 p.m., weight, 142 lbs.
May 14	80	66	77	No symptoms
May 15	80	70	78	No symptoms
May 16	77	68	76	No symptoms
May 17	80	70	78	No symptoms
May 18	80	69	75	No symptoms
May 19	81	66	79	No symptoms
May 20	78	70	77	No symptoms
May 21	80	66	76	Weight, 141¾ lbs.
May 22	80	65	77	No sympotms
May 23	78	67	75	No symptoms
May 24	80	70	77	No symptoms
May 25	79	68	75	No symptoms
May 26	80	66	78	Weight, 141.5 lbs.

tion of two individuals following an intravenous injection of 10 mgm. of thyroxin.

Following the injection there was a lag of approximately twenty-four hours before the first symptoms made their appearance. The rise in pulse rate recorded in the table and the statement of the principal symptoms does not adequately express the feelings of the two injected individuals. It is interesting to compare our tables 1 and 2 with the table in Carlson's (11) paper which details the results of continuous thyroid feeding to a normal, adult man. The symptoms induced by thyroid feeding and by intravenous injection of thyroxin are identical. Tachy-

cardia did not occur. Our experiment indicates that normal individuals react more violently to injections of thyroxin than do myxedematous or cretinous patients. So far as we are aware, 10 mgm. of thyroxin when given intravenously to such patients does not evoke toxic symptoms or at any rate to the extent observed by us. In the normal individual the thyroxin injected is superfluous and over and above that suited to the needs of the organism and induces untoward symptoms. Myxedematous

TABLE 4

Record of pulse and general condition of a 21-year old male individual following an intravenous injection of 10 mgm. of acetyl thyroxin

DATE 1924	PULSE RATE			REMARKS
	4–6 p.m., highest	7:00 a.m., lowest	Average	
May 6	79	59	73	
May 7	75	57	68	
May 8	78	60	72	
May 9	76	60	73	
May 10	77	56	70	
May 11	80	58	75	
May 12	76	56	74	
May 13	74	59	67	10 mgm. of acetyl thyroxin taken intravenously 4:15 p.m., weight, 173.5 lbs.
May 14	72	64	68	No symptoms
May 15	69	57	66	No symptoms
May 16	73	55	68	No symptoms
May 17	75	61	69	No symptoms
May 18	77	58	68	No symptoms
May 19	79	60	70	No symptoms
May 20	81	56	73	No symptoms
May 21	79	58	68	Weight, 174 lbs.
May 22	80	55	71	No symptoms
May 23	80	57	70	No symptoms
May 24	78	60	69	No symptoms
May 25	81	61	72	No symptoms
May 26	79	59	68	Weight, 174 lbs.

or cretinous individuals, on the contrary, demand large amounts of thyroid hormone and toxic effects are not produced following its administration in amounts such as used by us.

Intravenous injections of acetyl thyroxin into the same two individuals receiving thyroxin proved negative. The injections were given several weeks (approximately four) after the last thyroxin was taken. Tables 3 and 4 show clearly that acetyl thyroxin is physiologically inert when injected into mammals.

For oral administration the 2-mgm. tablets of the sodium salt of thyroxin, as prepared by E. R. Squibb & Sons under license from the University of Minnesota, were used. The acetyl derivative of thyroxin was administered orally in gelatine capsules. The tablets and capsules were taken about one hour before meal time and the pulse was taken five

TABLE 5

Record of pulse and general condition of 33-year old male individual fed acetyl thyroxin

DATE 1923	ACETYL-THYROXIN TAKEN	PULSE RATE			REMARKS
		4:00–6:00 p.m., highest	7:00 a.m., lowest	Average	
	mgm.				
November 23	None			80	Weight, 141 lbs.
November 24	None			81	
November 25	None			82	Total amount acetyl thyroxin taken 28 mgm.
November 26	None			79	
November 27	2	80	75	80	
November 28	2	82	76	80	
November 29	2	80	74	77	
November 30	2	82	76	79	
December 1	2	80	74	78	
December 2	2	78	68	73	
December 3	2	78	68	74	
December 4	2	80	68	74	
December 5	2	80	75	77	
December 6	2	80	73	72	
December 7	2	80	68	75	
December 8	2	78	68	72	
December 9	2	80	69	74	
December 10	None	78	73	74	
December 11	2	75	72	73	
December 12	None	80	70	74	
December 13	None	78	68	72	
December 14	None	80	69	76	
December 15	None	78	64	78	
December 16	None	80	65	78	
December 17	None	80	68	78	Weight, 141 lbs.

or six times a day beginning in the morning just before arising. Only the highest, lowest and average pulse are given for each day. After discontinuing the thyroxin the pulse was taken each day for a week in order to see if any rise occurred but these data are not included in the tables because in all cases the results were negative.

A survey of tables 5, 6, 7 and 8 shows quite clearly that acetyl thyroxin in doses of 2 mgm. per day for about two weeks has little if any effect upon the pulse rate. It was then decided that probably 2 mgm. per day

are insufficient to induce a physiological response. The reason this quantity was chosen was because the manufacturers of thyroxin state: "Concerning the clinical use of thyroxin, the following facts have been determined: a normal individual cannot take 2 milligrams of thyroxin a day continuously without developing symptoms of hyperthyroidism."

The acetyl thyroxin feeding was discontinued December 13. On January 10, one individual was fed 2 mgm. of pure thyroxin per day

TABLE 6

Record of pulse and general condition of 26-year old male individual fed acetyl thyroxin

DATE 1923	ACETYL-THYROXIN TAKEN	PULSE RATE			REMARKS
		Highest	Lowest	Average	
	mgm.				
November 23	None			84	Total amount acetyl thyroxin 28 mgm.
November 24	None			84	
November 25	None			76	
November 26	None	82	74	78	
November 27	2	82	76	80	
November 28	2	88	76	82	
November 29	2	88	74	86	
November 30	2	86	80	82	
December 1	2	90	80	85	
December 2	2	84	82	83	
December 3	2	86	84	84	
December 4	2	82	80	81	
December 5	2	84	80	82	
December 6	2	82	80	81	
December 7	2	84	80	82	
December 8	2	82	80	81	
December 9	2	84	80	82	
December 10	None	84	80	82	
December 11	2	82	80	81	
December 12	None	82	80	81	
December 13	None	84	80	82	
December 14	None	82	80	82	
December 15	None	82	80	81	
December 16	None	82	80	81	

for twelve days and then fed acetyl thyroxin in strong doses until a total of 76 mgm. of the acetyl derivative had been taken. The results are summarized in table 8 and show clearly that 24 mgm. of thyroxin and 76 mgm. of the acetyl derivative taken in strong doses continually (except for three days) for twenty-six days exerts no influence upon the pulse rate.

Our negative findings with acetyl thyroxin on man are in agreement with those of Kendall who used this substance on dogs, and with our results on intravenous injection. We had anticipated negative results following administration of the acetyl thyroxin but the consistently negative findings in the case of the orally administered thyroxin was a surprise.

TABLE 7

Record of pulse and general condition of 21-year old male individual fed acetyl thyroxin

DATE 1923	ACETYL-THYROXIN TAKEN	PULSE RATE			REMARKS
		Highest	Lowest	Average	
	mgm.				
November 23	None			72	Weight, 170 lbs.
November 24	None			72	
November 25	None			72	Total amount of acetyl thyroxin taken, 30 mgm.
November 26	None			73	
November 27	2	78	64	73	
November 28	2	78	66	72	
November 29	2	78	65	74	
November 30	2	70	68	70	
December 1	2	74	60	66	
December 2	2	74	62	70	
December 3	2	72	60	72	
December 4	2	72	60	69	
December 5	2	72	60	69	
December 6	2	70	59	66	
December 7	2	67	56	66	
December 8	2	80	70	72	
December 9	None		65	70	
December 10	None	72	62	67	
December 11	2	72	58	68	
December 12	2	72	64	69	
December 13	2	76	72	74	
December 14	None	73	70	71	
December 15	None	72	68	70	
December 16	None	70	66	68	
December 17	None	71	68	70	
December 18	None	72	66	69	Weight, 170 lbs.

The data on thyroxin feeding given in tables 9, 10 and 11 clearly indicate that this substance when given orally in the quantity used by us exerts little effect upon the pulse rate of normal, healthy individuals. Table 11 is of particular interest because the individual represented had never received any acetyl thyroxin or thyroxin previous to his first dose on February 15, whereas the other three individuals had previously (about

TABLE 8

Record of pulse and general condition of 21-year old male individual fed sodium salt of thyroxin and acetyl thyroxin

DATE 1924	THYROXIN TAKEN	PULSE RATE			REMARKS
		Highest	Lowest	Average	
	mgm.				
January 8	None			66	Total amount of thyroxin taken, 24 mgm. Acetyl thyroxin, 76 mgm.
January 9	None			65	
January 10	2	72	66	69	
January 11	2	70	65	68	
January 12	2	72	68	70	Weight, 170 lbs.
January 13	2	80	70	76	
January 14	2	78	72	75	
January 15	2	75	66	71	
January 16	2	120	69	88	High pulse taken after brisk walk
January 17	2	75	70	72	
January 18	2	112	68	83	High pulse taken after brisk walk
January 19	2	80	71	76	
January 20	2	76	62	71	
January 21	2	81	64	73	
	ACETYL-THYROXIN				
January 22	4	75	69	71	
January 23	4	68	60	65	
January 24	4	70	66	68	
January 25	4	70	60	65	
January 26	4	78	68	72	
January 27	4	75	60	68	
January 28	6	74	62	66	
January 29	6	72	58	67	
January 30	6	80	60	71	
January 31	6	78	62	70	
February 1	None	72	64	68	
February 2	None	75	66	71	
February 3	None	77	65	70	
February 4	8	80	67	71	
February 5	8	80	67	74	
February 6	8	79	68	73	
February 7	4	80	60	69	
February 8	None			70	Weight, 174 lbs.
February 9	None			71	

one month) taken 28 mgm. of acetyl thyroxin. The data in table 11 rule out the possibility of any tolerance for thyroxin having developed, thus

TABLE 9

Record of pulse and general condition of 33-year old male individual fed sodium salt thyroxin

DATE 1924	THYROXIN TAKEN	PULSE RATE			REMARKS
		2:00 p.m., highest	7:00 a.m., lowest	Average	
	mgm.				
January 8	None	74	74	74	Total amount of thyroxin taken, 112 mgm.
January 9	None	76	65	70	
January 10	2	78	72	75	
January 11	2	80	72	78	
January 12	2	75	69	74	Weight, 138.75 lbs. on this date
January 13	2	78	73	75	
January 14	2	90	72	80	
January 15	2	83	70	75	
January 16	2	83	72	88	
January 17	2	81	72	72	
January 18	2	82	71	83	
January 19	2	81	75	76	
January 20	2	83	76	71	
January 21	2	112	74	73	112 at 2:00 p.m.
January 22	4	93	72	71	90 at 6:00 p.m.
January 23	4	83	72	65	74 at 10:00 p.m.
January 24	4	97	55	68	Pulse irregular
January 25	4	91	74	65	
January 26	4	94	72	72	
January 27	4	90	74	68	
January 28	6	102	65	80	Pulse down to 72 at 6:00 p.m.
January 29	6	100	70	80	
January 30	6	97	70	78	Pulse down to 72 at 6:00 p.m.
January 31	9	85	70	83	Crystalline thyroxin taken
February 1	None	97	63	78	
February 2	None	84	72	76	
February 3	None	87	73	78	
February 4	8	102	75	78	Thyroxin taken at 5:30 p.m.
February 5	8	90	74	78	
February 6	8	79	74	78	
February 7	5	83	72	78	Weight, 141 lbs. on this date
February 8	8	75	66	76	Crystalline thyroxin taken
February 9	None	78	72	76	

accounting for the more or less negative findings. None of the persons taking thyroxin by mouth reported any ill effects whatever. Dizziness, headache, nervousness or other symptoms were not noted.

Two thyroxin-fed individuals report a loss of weight of three and four pounds respectively, and one, a two and one-quarter pound gain. The

TABLE 10

Record of pulse and general condition of 26-year old male individual fed sodium salt of thyroxin

DATE 1924	THYROXIN TAKEN	PULSE RATE			REMARKS
		Highest	Lowest	Average	
	mgm.				
January 8	None	84	80	82	Total amount thyroxin taken, 106 mgm.
January 9	None	82	78	80	
January 10	2	84	80	82	
January 11	2	90	82	86	
January 12	2	80	78	79	Weight, 169 lbs.
January 13	2	80	76	79	
January 14	2	94	80	85	
January 15	2	102	80	87	80 at 11:00 a.m., 102 at 8:00 p.m., 80 at 11:00 p.m.
January 16	2	82	74	79	
January 17	2	80	78	79	
January 18	2	82	78	80	
January 19	2	80	70	76	
January 20	2	76	74	75	
January 21	2	91	90	91	
January 22	4	105	80	88	
January 23	4	84	74	80	
January 24	4	82	80	81	
January 25	4	84	80	82	
January 26	4	94	76	83	
January 27	4	80	72	77	
January 28	6	90	82	86	
January 29	6	90	86	89	
January 30	6	96	80	87	
January 31	8	90	70	81	
February 1	None	84	80	83	
February 2	None	82	80	81	
February 3	None	84	80	82	
February 4	8	91	84	87	
February 5	8	86	80	83	Crystalline thyroxin taken
February 6	8	82	80	81	
February 7	8	84	82	83	Weight, 165 lbs.
February 8	None	84	80	82	
February 9	None	82	80	81	
February 10	None	84	80	82	
February 11	None	90	82	86	

individual reporting the four-pound loss during an interval of twenty-five days states that his weight fluctuates considerably, and considers the weight loss of little significance. On the other hand, the individual

reporting the three-pound loss states that his weight remains fairly constant and varies little. The weight loss of this individual occurred during an interval of eighteen days. The third individual reporting a gain in weight states that his weight varies considerably. The fourth man receiving 38 mgm. of thyroxin and 76 mgm. of acetyl thyroxin gained four pounds and states that his weight is not subject to variation. It is unfortunate that daily weighings were not made. Kendall (1) remarks that patients receiving group A thyroid constituents (thyroxin) and presenting very marked physiological response such as greatly in-

TABLE 11

Record of pulse and general condition of 19-year old male individual fed sodium salt of thyroxin

DATE 1924	THYROXIN TAKEN	PULSE RATE			REMARKS
		6:00 p.m., highest	7:30 a.m., lowest	Average	
	mgm.				
February 15	8	76	71	72	Weight, 146 lbs.
February 16	8	80	59	80	Total amount thyroxin taken, 104 mgm.
February 17	8	76	70	75	
February 18	8	85	65	78	
February 19	8	78	50	63	
February 20	8	80	55	67	
February 21	8	81	59	75	
February 22	8	77	65	70	
February 23	8	80	65	72	
February 24	8	77	69	72	
February 25	8	78	66	72	
February 26	8	91			Only one pulse rate taken
February 27	8	71	69	70	
February 28	None	79	70	74	
February 29	None	70	68	69	
March 1	None			71	
March 2	None			73	
March 3	None			71	Weight, 143 lbs.
March 4	None			72	

creased pulse rate (150 per minute) and nervous symptoms, may or may not show loss of weight.

Discussion. It is generally stated that thyroxin represents the active principle of the thyroid hormone, and a considerable amount of clinical and experimental data, especially basal metabolism tests, support this contention. Clinical work on the treatment of cretinism and myxedema with intravenous injections of thyroxin shows clearly that these conditions of hypothyroidism can be successfully treated with this substance. Myxedematous patients at the Mayo Clinic are treated with thyroxin in a

quantitative manner. If the metabolic rate is 40 per cent below normal they are brought to normal by the administration of 20 mgm. of thyroxin. Plummer has shown that a quantitative relationship exists between thyroxin and the basal metabolic rate. The administration of 1 mgm. of thyroxin to an adult weighing approximately 150 pounds increases the basal metabolic rate 2 per cent. The administration of 2 mgm. increases it 4 per cent; the administration of 10 mgm., 20 per cent. The experimental work on mammals shows that thyroxin influences the metabolic rate of these forms qualitatively in the same fashion as thyroid extract or tissue.

We were greatly surprised to find that the sodium salt of thyroxin and the pure crystalline substance were practically inactive when taken orally by normal individuals. So far as we are aware, this fact has not been reported in the literature. Our experiments indicate that thyroxin should be administered to myxedematous and cretinous patients intravenously if thyroid-like effects are to be produced. It will be recalled that the physiologic activity of thyroid tissue or extract is not diminished by passage through the alimentary canal, thus indicating that thyroxin differs somewhat from the thyroid hormone as it actually exists within the gland.

Careful studies on the comparative physiologic activity of thyroid and thyroxin are few, but two being known to the writers. Utilizing the acetonitrile test, Hunt (12), in a very extensive series of experiments, attempted to determine if thyroxin when fed to mice has an action qualitatively and quantitatively like that of thyroid, and if thyroxin in proportion to its iodine content differs in the degree of its activity from thyroid. His tables show that thyroxin has the qualitative activity of thyroid; its quantitative activity also being vastly greater than that of any other iodine-containing substance except the thyroid. But in not a single instance among the numerous experiments was the thyroxin, in proportion to its iodine content, as active as any of the normal adult thyroids used. Hunt's protocols show that thyroid tissue, in proportion to its iodine content, is about one and one-half times as active as thyroxin, when the latter was given either orally or intravenously. It is interesting to note that the thyroxin when given orally to mice exerts a qualitative effect like thyroid in raising the resistance of these animals to acetonitrile. It seems that in mice the thyroxin is either not destroyed in the alimentary canal as is probably the case in man, or else the increase in the resistance of mice to acetonitrile after thyroid or thyroxin administration depends upon some other chemical grouping in the hormone than that primarily responsible for the unique effect of this substance upon mammalian metabolism.

Cameron and Carmichael (13) using as a criterion the retarding effects of thyroid administration upon the growth of rats, found thyroid to be from two to four times as active as thyroxin when fed in equi-iodine dosage. They suggested that probably the thyroxin was destroyed in the alimentary tract to a greater extent than was the thyroid. Hunt reports, however, that although the protective action of thyroxin to the lethal effects of acetonitrile developed more rapidly when the drug was given intravenously than when fed, the degree of protection ultimately attained was not materially greater. However, there is some additional indirect evidence that thyroxin when given orally is less active than when administered intravenously; for instance Snell, Ford and Rowntree (14) state "we have had some difficulties with administration of thyroxin by mouth, but we have succeeded in holding some of the patients (cretins) at a normal metabolism by oral administration." The authors had no trouble whatever when the drug was given intravenously. The graph in figure 3 of their paper is suggestive in this connection.

The fact that both thyroxin and its acetyl derivative are active in inducing amphibian transformation, whereas only the thyroxin shows physiologic activity when administered to mammals is excellent evidence that amphibians and mammals differ considerably in regard to the nature of the chemical groupings within the thyroid hormone to which they respond. The substitution of acetyl for the hydrogen of the imino of thyroxin completely destroys its physiologic activity insofar as the metabolic rate of mammals is concerned, but this substitution apparently has no influence upon the iodine-containing group in the molecule responsible for amphibian metamorphosis.

SUMMARY

1. The experiments were designed to test the view that the physiologic responses of amphibians and mammals to thyroid administration depend upon different chemical groupings within the hormone.

2. Both thyroxin and its acetyl derivative are highly active agents in inducing the metamorphosis of thyroidectomized and hypophysectomized tadpoles when either fed or injected.

3. Thyroxin when given intravenously to normal, adult men exerts a profound physiologic response, comparable to that evoked by excessive thyroid feeding.

4. Oral administration of thyroxin gave practically negative results despite the large dosage employed. Thyroxin is apparently destroyed within the alimentary canal of man.

5. Acetyl thyroxin when intravenously injected into normal individuals exerts no physiologic response. Oral administration of this substance likewise proved negative.

6. Chemically the only difference between thyroxin and the acetyl derivative is the substitution of acetyl for the hydrogen of the imino group.

7. Amphibian metamorphosis depends upon a peculiar organic iodine complex as shown by the effect of acetyl thyroxin, iodotyrosin, iodoserumalbumin, iodoserumglobulin and iodized casein upon these forms.

8. The effect of thyroid upon mammalian metabolism is apparently unique and is not induced by other organic iodine compounds which metamorphose amphibians. The metabolic responses of mammals and the metamorphic responses of amphibians to thyroid are hardly to be compared as they are apparently due to quite different chemical groupings within the hormone although the iodine seems essential for the activity of both chemical groups, i. e., the group responsible for mammalian metabolism and the group for amphibian metamorphosis.

BIBLIOGRAPHY

(1) KENDALL: Collected papers of the Mayo Clinic, 1915, 393.
(2) SWINGLE: Science, 1922, lvi, 720.
(3) SWINGLE: Journ. Exper. Zoöl., 1923, xxxvii, 219.
(4) SWINGLE: Biol. Bull., 1923, xlv, 229.
(5) SWINGLE: Anat. Rec., 1924, xxvii, 221.
(6) OSWALD: Pflügers Arch. 1916–17, clxvi, 169.
(7) HELLIN: Arch. f. Exper. Path. u. Pharm., 1897, xl, 121.
(8) BLUM: Münch. med. Wochenschr., 1898, xlv, 270.
(9) STROUSE AND VOEGTLIN: Journ. Pharm. Exper. Therap., i, 123.
(10) KENDALL: This Journal, 1919, xlix, 136.
(11) CARLSON, ROOKS AND MCKIE: This Journal, 1912, xxx, 129.
(12) HUNT: This Journal, 1923, lxiii, 257.
(13) CAMERON AND CARMICHAEL: Journ. Biol. Chem., 1920, xlv, 69.
(14) SNELL, FORD AND ROWNTREE: Journ. Amer. Med. Assoc., 1920, lxxv, 515.